THE ANALYST

Analytical Methods Committee

REPORT PREPARED BY THE MEAT PRODUCTS SUB-COMMITTEE

Nitrogen Factors for Pork

THE Analytical Methods Committee has received the following Report from its Meat Products Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The Meat Products Sub-Committee was appointed by the Analytical Methods Committee in 1955 to succeed the Meat Extracts Sub-Committee, which had been in existence since 1948 under the chairmanship of Mr. G. Taylor. It was reconstituted with wider terms of reference than its predecessor, and its new title indicated the widening of the scope of its work.

Its first Chairman was Dr. H. G. Rees, who had been Honorary Secretary of the old Sub-Committee, but, on his resignation in 1956, he was succeeded by Dr. S. M. Herschdoerfer; its constitution is as follows: Mr. S. Back, Mr. P. O. Dennis, Mr. J. R. Fraser, Dr. R. A. Lawrie, Dr. A. McM. Taylor and Mr. E. F. Williams (deputy, Mr. H. C. Hornsey), with Dr. C. H. Tinker as Secretary. The following have also served on the Sub-Committee: Miss E. M. Chatt (October, 1955, to June, 1960), Mr. C. D. Essex (June, 1955, to January, 1960) and Dr. H. Amphlett-Williams (June, 1955, to January, 1961). The Terms of Reference are—

> "(*a*) The determination of the meat content of products containing meat; (*b*) the determination of the constituents of meat and meat products.
>
> NOTE—The term 'meat products' to include hydrolysed protein and, if found necessary, fish pastes."

In the first instance, the Sub-Committee reviewed the alternative methods for the determination of the meat content of manufactured products—namely, that of Stubbs and More,[1] in which the nitrogen content is determined, and that of Osman Jones, based on the determination of starch content. The Sub-Committee considers Stubbs and More's method to be more reliable and suggests that, as a further check, a determination of starch by the method of Fraser and Holmes[2] may be used. There was evidence that the nitrogen factors published by Stubbs and More in 1919 for the different types of meat (pork, beef, mutton, etc.), or even the modified factor of 3·6 for pork (recommended by the Analytical Methods Committee in 1940),[3] were not altogether valid for present-day use. The Sub-Committee therefore reviewed all data relating to the determination of nitrogen factors published over the last 30 years.

Jackson and Jones,[4] on the basis of 21 analyses of lean pork, including 10 of "mixed meat," derived an unweighted mean for the water-to-protein ratio of 3·4 to 1, corresponding, on the basis of a nominal ash content of 1 per cent., to 3·60 per cent. of nitrogen in the fat-free meat. Steiner,[5] quoting results from a private communication, states that " . . . an examination of the analytical data for various cuts of pork has shown that the protein percentage

in the fat-free portion of the meat is distributed approximately normally, with mean value 21·9 per cent." (equivalent to 3·50 per cent. of nitrogen) "and standard deviation of 2·76 per cent." Reith, Hofsteede and Langbroek[6] examined the meat from 19 Dutch animals, all meat muscle being "cut out, divided into 50-g pieces and mixed in the same quantitative relation as exists in the animal." The average nitrogen factor from these analyses was 3·40.

In analyses by Marshall,[7] the nitrogen content (on a fat-free basis) of the whole of the lean meat from one side of pork ". . . calculated by proportion was 3·39 per cent.; a higher figure of 3·43 per cent., which is less accurate, is obtained by direct averaging of the analyses." In his second series of analyses, for which another carcass was used, ". . . the nitrogen content of the lean meat on a fat-free basis, calculated both by proportion and by direct averaging, was 3·43 per cent." A circular[8] issued by the American Meat Institute Foundation quotes a large number of figures; these, however, refer partly to analyses reported in 1928 and include many types of meat not used in Britain for manufactured products.

Experimental and results

In view of the paucity of published data, the Sub-Committee appealed to various meat product manufacturers and meat research organisations in this country and abroad for information on the analyses of meat products, with particular reference to the nitrogen contents of pig carcasses and different types of cuts. Nearly 1200 results, ranging from 3·0 to 4·0 per cent. and including values for individual muscles, named cuts and unspecified mixtures of pork meat, were submitted to the Sub-Committee.[9] These results, together with corresponding values from the literature, are shown in Fig. 1, from which it is apparent that there are considerable differences between the nitrogen contents of the fat-free meat in the different cuts of the animal.

The Sub-Committee considered the possibility of calculating the value for a nitrogen factor for the whole carcass from these figures, but thought this to be too dependent on arbitrary assumptions. Apart from the uncertainty associated with such assumptions, a further limitation arises from the fact that most of the analytical results were obtained from small samples of the relatively large cuts of meat involved, thereby introducing the possibility of sampling errors. In view of these difficulties, two particular series of tests were carried out, at the request of the Sub-Committee, in which the entire cuts of meat were comminuted and thoroughly mixed before sampling. These tests were planned to give an automatic weighting of the analytical figures in relation to the proportionate amounts of the cuts in the carcass.

In the first series of tests, 18 carcasses were divided into left and right sides. In each instance, one side was completely boned out, very finely comminuted, mixed and used as sample for determining the average nitrogen content of all the edible meat; the other side was divided into shoulder, middle and leg cuts, each of which was boned out, comminuted and analysed. Gristle, fat, rinds, ears, snouts, tails, gullets, tongues and offal were excluded, so that only the carcass meat—the so-called "usable meat"—was employed for the analytical work. Each sample was analysed for fat, nitrogen and water contents. A single sample from each mix was taken and analysed in duplicate, each observation recorded being the arithmetical average of these two analyses. The results from the analyses of 18 carcasses are shown in Table I.

Table I

Nitrogen contents found in pork meat from 18 carcasses

Cut	Average nitrogen content (fat-free basis), %	Standard deviation of a single observation, %	Coefficient of variation, %
Side A—			
Shoulder	3·37	0·08	2·3
Middle	3·66	0·07	1·9
Leg	3·52	0·06	1·7
Side B—			
All edible meat from whole side	3·45	0·075	2·2

In the second series of tests, in order to determine the average nitrogen content of all edible meat, *i.e.*, lean and fatty tissue, but not offal, one side from each of a further 35 carcasses was completely boned out, and the meat was very finely comminuted and mixed. Two samples of each lot, *i.e.*, each carcass, were taken, and a single analysis was made on each sample, each

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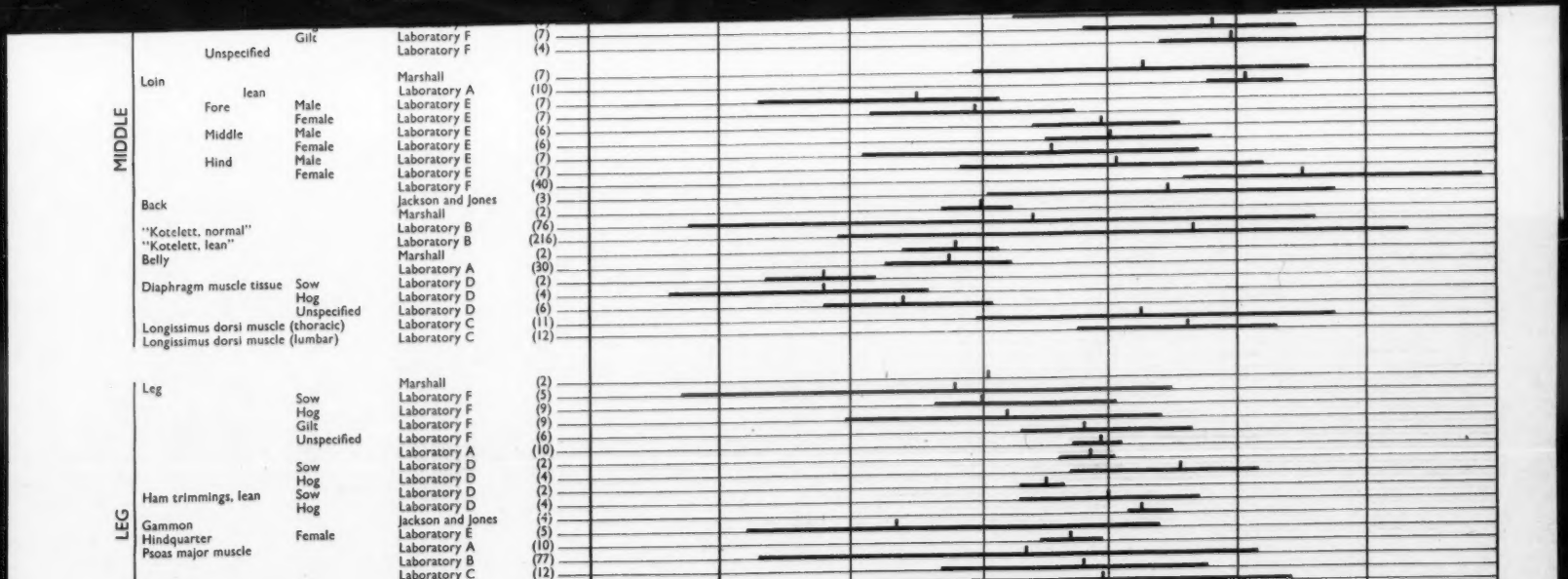
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Middle
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Hind
Male
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Female
Laboratory E (7)
Laboratory F (40)
Back
Jackson and Jones (3)
Marshall (2)
"Kotelett, normal"
Laboratory B (76)
"Kotelett, lean"
Laboratory B (216)
Belly
Marshall (2)
Laboratory A (30)
Diaphragm muscle tissue
Sow
Laboratory D (2)
Hog
Laboratory D (4)
Unspecified
Laboratory D (6)
Longissimus dorsi muscle (thoracic)
Laboratory C (11)
Longissimus dorsi muscle (lumbar)
Laboratory C (12)
Leg
Marshall (2)
Sow
Laboratory F (5)
Hog
Laboratory F (9)
Gilt
Laboratory F (9)
Unspecified
Laboratory F (6)
Laboratory A (10)
Sow
Laboratory D (2)
Hog
Laboratory D (4)
Ham trimmings, lean
Sow
Laboratory D (2)
Hog
Laboratory D (4)
Gammon
Jackson and Jones (4)
Hindquarter
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Laboratory E (5)
Psoas major muscle
Laboratory A (10)
Laboratory B (77)
Laboratory C (12)

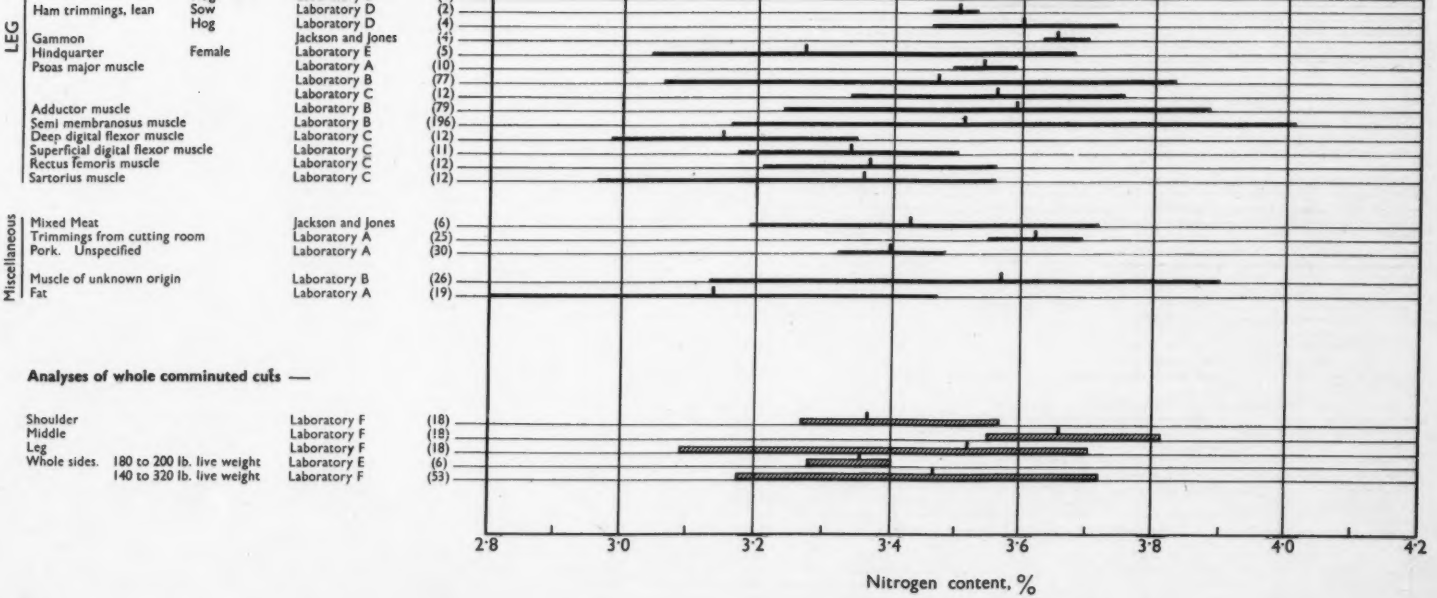

Fig. 1. Nitrogen contents of various cuts of meat. Horizontal lines represent the range of nitrogen contents, short vertical lines indicate the average values

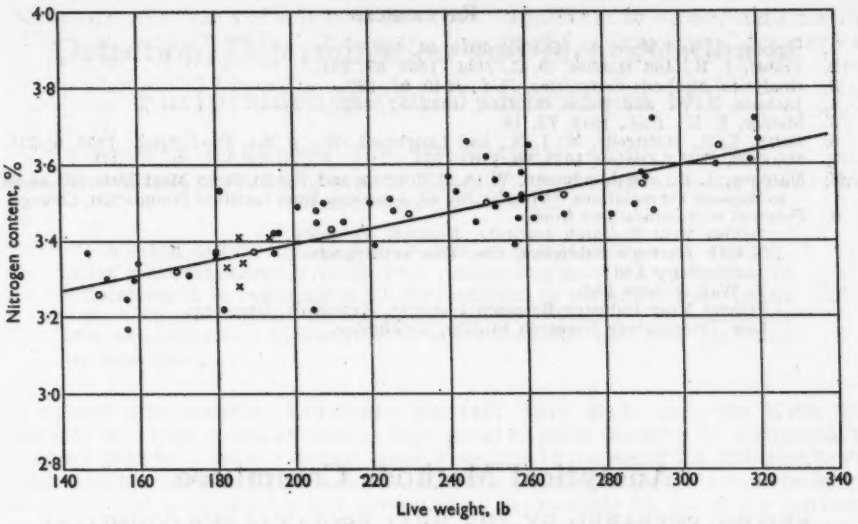

Fig. 2. Relationship between nitrogen content of fat-free meat and live weight of animal: ●, results found by laboratory F; ×, results found by laboratory E; ○, average results (see Table II)

observation recorded being the arithmetical average of these two determinations. The overall average nitrogen content (fat-free basis), for the 53 carcasses was 3·47 per cent., with a standard deviation of individual observations of 0·12 per cent.

The series of results forming the basis of this assessment was obtained from a number of pigs ranging in weight from 147 to 320 lb. A significant regression of the nitrogen content (fat-free basis) on live weight was apparent (see Fig. 2). Another laboratory, examining pigs of live weight 180 to 200 lb, found an average value of 3·36 for 6 carcasses (whole meat) as the nitrogen factor. These results are also shown in Fig. 2 and are seen to be on the same regression line. The relationship between the nitrogen factor and the live weight is summarised in Table II.

TABLE II

RELATIONSHIP BETWEEN NITROGEN FACTOR AND LIVE WEIGHT

These results are for the total edible meat from 59 carcasses

Live weight of animal, lb	Nitrogen content (fat-free basis), %
140 to 159	3·26
160 to 179	3·32
180 to 199	3·37
200 to 219	3·43
220 to 239	3·47
240 to 259	3·50
260 to 279	3·52
280 to 299	3·55
300 to 320	3·65

RECOMMENDATION

After due consideration of the types of meat normally used in the manufacture of comminuted products, and of the use of pigs of varying live weight, the Sub-Committee recommends an average nitrogen factor of 3·45 as the best compromise for general use.

References

1. Stubbs, G., and More, A., *Analyst*, 1919, **44**, 125.
2. Fraser, J. R., and Holmes, D. C., *Ibid.*, 1958, **83**, 371.
3. Analytical Methods Committee, *Ibid.*, 1940, **65**, 257.
4. Jackson, F. W., and Jones, O., *Ibid.*, 1932, **57**, 562.
5. Steiner, E. H., *Ibid.*, 1948, **73**, 15.
6. Reith, J. F., Hofsteede, M. J. N., and Langbroek, W., *J. Sci. Food Agric.*, 1955, **6**, 317.
7. Marshall, C. R., *Analyst*, 1955, **80**, 776.
8. Maroney, J. E., and Landmann, W. A., "Moisture and Fat in Fresh Meat Materials as Related to Sausage Formulations," *Circular No.* 49, American Meat Institute Foundation, Chicago, 1959.
9. Personal communications from—
   Danish Meat Research Institute, Roskilde, Denmark.
   N.V.H. Hartog's Fabrieken, Oss, The Netherlands.
   J. Sainsbury Ltd.
   T. Wall & Sons Ltd.
   Federal Meat Industry Research Institute, Kulmbach, Germany.
   Low Temperature Research Station, Cambridge.

Analytical Methods Committee

REPORT PREPARED BY THE MEAT PRODUCTS SUB-COMMITTEE

Nitrogen Content of Rusk Filler

THE Analytical Methods Committee has received the following Report from its Meat Products Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

Report

The Sub-Committee responsible for the preparation of the Report was constituted as follows: Dr. S. M. Herschdoerfer (Chairman), Mr. S. Back, Mr. P. O. Dennis, Mr. J. R. Fraser, Dr. R. A. Lawrie, Dr. A. McM. Taylor and Mr. E. F. Williams (deputy, Mr. H. C. Hornsey), with Dr. C. H. Tinker as Secretary.

Although various types of cereal filler, *e.g.*, rusk, bread and rice, can be used in the manufacture of sausages and some other meat products, the general practice nowadays is to use rusk, and this Report deals only with the correction to be made for nitrogen in this type of filler. No correction for nitrogen is made when the filler consists of potato starch* or corn flour (*i.e.*, maize starch).

In 1952, the Analytical Methods Committee[1] recommended that, in calculating the results of analyses, a figure corresponding to 2 per cent. of the dry carbohydrate *plus* the crude cellulose should be deducted from the result for total nitrogen to allow for nitrogen present in the cereal filler. However, in view of the wide range of nitrogen contents of flours, this figure of 2 per cent. would appear to correspond to rusk flour of the lowest protein content. Values for analyses of rusks recently submitted by rusk manufacturers indicate nitrogen contents ranging from 1·9 to 2·6 per cent. (calculated on dry carbohydrate).

In this event, the Sub-Committee recommends as the best compromise a figure of 2·3 per cent. as the correction for nitrogen in the rusk filler; this figure corresponds to the average composition of culinary flour. The Sub-Committee also recomends that this figure should be reviewed periodically, in view of changes that occur in milling practices.

Reference

1. Analytical Methods Committee, *Analyst*, 1952, **77**, 543.

* This is known in the trade as farina and should not be confused with potato flour.

Detection, Determination and Identification of Furfuraldehyde in Hydrocarbon Oil

By R. B. HARRISON, J. F. PALFRAMAN and B. A. ROSE

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A colour test for detecting furfuraldehyde in hydrocarbon oil, a quantitative spectrophotometric method for determining from 1 to 10 p.p.m. of furfuraldehyde in hydrocarbon oil (by formation of the addition compound with aniline acetate) and a gas-chromatographic identification of furfuraldehyde after extraction from hydrocarbon oil as the aldehyde - bisulphite compound are described.

In accordance with Statutory Instrument No. 1127, 1948, made under the Motor Spirit (Regulation) Act, 1948, chemical markers were added to petrol intended for commercial use. The markers included a red dye so that visual inspection of the petrol was sufficient to show for which class of road users it was intended. Petrol in the tank of a vehicle could be sampled by the police, and a colorimetric test performed at the roadside for one of the markers; if this test was positive, a sample was taken for further tests in the laboratory to establish the presence of "red" petrol. Quantitative work was not necessary, and although some was carried out no reports on it were published. The same problem is now posed by "diesel oil" and "gas oil," which are often identical in composition, but have different uses. Diesel oil, or DERV, is used as a fuel for diesel-engined road vehicles and, as such, attracts duty as a road fuel; gas oil is used in gas manufacture and as a fuel for stationary diesel-engined machinery and therefore attracts no road-fuel duty. It has been decided to mark oils of this type in order to prevent their misuse as road fuel.

The markers had to be selected with care, and five requirements had to be satisfied—

(*i*) A rapid and simple colorimetric test, with the minimum of apparatus, for use at the roadside.

(*ii*) An accurate quantitative method of determination for laboratory use.

(*iii*) Positive identification of the added chemical.

(*iv*) Each marker added must not interfere with the detection, determination and identification of any other added marker.

(*v*) The marker must have sufficient solubility in a hydrocarbon solvent to permit concentrates to be prepared in volumes convenient to mark several thousands of gallons of oil at a time.

Furfuraldehyde fulfilled these conditions and was chosen as one of the markers. It should be mentioned here that furfuraldehyde is used in the refining of heavy oils, but the recovery processes used by the oil companies are highly efficient. It is in the manufacturers' interest to remove traces of furfuraldehyde, as these cause discoloration due to oxidation. In more than twenty samples of gas and diesel oils obtained from various different companies and depots throughout the United Kingdom, furfuraldehyde was found in only one, and then at the level of 0·1 p.p.m.

DETECTION OF FURFURALDEHYDE

An ideal roadside test is one in which an impregnated paper is used, thereby obviating the necessity for glassware and solutions. Aniline acetate was found to be the most sensitive colour reagent for furfuraldehyde, but was unsuitable for impregnating paper, owing to its instability in the absence of excess of acetic acid. However, if spots of the aniline acetate reagent solution were placed on the strip of filter-paper just before immersion for a few seconds in an oil containing furfuraldehyde, a red colour was produced; this colour reached maximum intensity in about 15 seconds and then faded. By this method, 2·5 p.p.m. of

furfuraldehyde were just detectable in a pale oil and 10 p.p.m. in a dark oil. As the required limit was 1 p.p.m. in pale or dark oils, this technique was abandoned in favour of that described below.

Method

Reagent—

Aniline acetate solution—Dissolve 100 ml of analytical-reagent grade aniline in 900 ml of glacial acetic acid. Store the solution in a dark-coloured bottle.

Procedure—

Place 1 ml of aniline acetate solution and 15 ml of the suspect gas oil in a test-tube (6 inches × 0·75 inch), invert the tube three times, allow the layers to separate, and observe the colour of the lower layer. A bright red colour indicates the presence of furfuraldehyde; a negative test results in a yellow-brown lower layer. With dark-coloured oils, the result is sometimes easier to see if the tube is emptied, when some of the acetic acid phase adheres to the glass, leaving a red film if furfuraldehyde is present. With this test, concentrations of furfuraldehyde down to 1 p.p.m. can be detected in pale or dark oils, equivalent to a dilution of one part of marked oil with nine parts of unmarked oil. The lower limit in a pale oil is 0·2 p.p.m., which results in a pink colour rather than bright red. The colours produced persist for several minutes.

DETERMINATION OF FURFURALDEHYDE

Several methods for determining furfuraldehyde in oil have been described,[1 to 5] and we have modified that of Milner and Liederman[1] to give a rapid method having the required accuracy and involving use of the minimum amount of sample. The method proposed is designed for the range 1 to 10 p.p.m. of furfuraldehyde, but can be extended to determine higher concentrations by dilution of the oil containing the furfuraldehyde.

Difficulty was experienced in extracting the furfuraldehyde completely from the oil, yet maintaining the volume of solution sufficiently small for the production of a colour having an intensity suitable for spectrophotometric determination. It was therefore decided to measure the colour of the Schiff's base produced in the reversible reaction between furfuraldehyde and aniline acetate *in situ* in the oil.

As aniline acetate in glacial acetic acid is immiscible with gas oil, a diluent was needed, and toluene was used instead of benzene on account of the toxicity of benzene vapour. When the diluted gas oil was mixed with the aniline acetate solution, a red colour developed, increasing to a maximum and then fading; this process was followed with a spectrophotometer. The maximum optical density attained was a function of the concentration of furfuraldehyde in the gas oil, and the time within which the maximum was reached was also dependent on the concentration of furfuraldehyde, being about 2 to 3 minutes for concentrations of 1 to 10 p.p.m. and 1 to 2 minutes for concentrations below 1 p.p.m.

Method

Reagents—

Standard furfuraldehyde solution—From a stock solution containing 0·5 per cent. w/v of freshly distilled furfuraldehyde in gas oil prepare a solution containing 10 p.p.m. of furfuraldehyde.

Aniline acetate solution—Prepare as described above.

Procedure—

By pipette, place 5·0 ml of gas oil in a 25-ml calibrated flask, and add 7 to 8 ml of toluene. (Larger aliquots of gas oil cannot be used, owing to immiscibility with the reagent.) From a pipette having an automatic filler add 10·0 ml of aniline acetate solution, and dilute to the mark with toluene. Invert the flask, shake to mix the contents, and rapidly transfer a few millilitres to a parallel-sided 1-cm cell of a Unicam SP600 spectrophotometer or similar instrument. Use as blank solution 5·0 ml of the same gas oil *plus* 20·0 ml of toluene. Set the wavelength scale at 520 mμ, follow the optical density until it attains the maximum, and record this figure. Plot a graph of optical density against concentration of furfuraldehyde from measurements made on pairs of aliquots, one being treated with reagent and the other diluted with toluene as blank.

Discussion of the method

The aniline acetate solution exhibits slight absorption at 520 mμ, the concentration used in the procedure described above giving, when fresh, an optical-density reading of 0·004 (equivalent to 0·07 p.p.m. of furfuraldehyde). This reagent darkens to a yellow colour within 24 hours and must therefore be freshly prepared each day, so that, when the blank value just mentioned is used, the correction is within the limits required. The use of the same amount of gas oil in the blank automatically compensates for any colour in the oil.

Limits—

If furfuraldehyde is dissolved in toluene instead of gas oil and toluene is used as blank, the graph of optical density against concentration of furfuraldehyde in the range 1 to 10 p.p.m. is linear and passes close to the origin. With a solution of furfuraldehyde in a gas oil and an equivalent amount of the same gas oil in the blank, a similar plot gives a graph following the above within 0·1 p.p.m. up to 6 p.p.m. of furfuraldehyde and then deviating from it, the maximum deviation being 0·7 p.p.m. at the 10 p.p.m. level. This deviation is probably caused by the difference in the effects of gas oil and toluene on the equilibrium of the reaction, the gas oil appearing to inhibit the decomposition of the Schiff's base. To attain an accuracy within 0·1 p.p.m., therefore, the range 1 to 6 p.p.m. of furfuraldehyde should be used; dilution of the gas oil with an equal volume of toluene will bring any result between 6 and 10 p.p.m. within this range. Repeatability is within 0·1 p.p.m. in the range 1 to 10 p.p.m. of furfuraldehyde.

Stability of furfuraldehyde in oil—

Twelve gas oils having different colours and compositions from various sources were "dosed" to the level of 10 p.p.m. of furfuraldehyde; they were analysed immediately and at fortnightly intervals for 3½ months to check the stability of the marker in the oils at this concentration. Some of the results are shown in Table I, from which it can be seen that the concentration of furfuraldehyde does not vary significantly over 3 months, by which time it is estimated that any particular batch of oil leaving a refinery will have been used. The results also show the effects of the gas oils on the equilibrium, the apparent initial concentrations of furfuraldehyde being in the range 9·3 to 10·1 p.p.m. It must be emphasised that these results are outside the range of greatest accuracy and were intended only to check the stability of the marker at this concentration.

Table I

Variation in concentration of furfuraldehyde with time

Oil No.	Concentration of furfuraldehyde found— initially, p.p.m.	after 14 days, p.p.m.	after 3½ months, p.p.m.
1	10·0	10·2	10·8
2	9·5	10·1	10·6
3	9·6	9·9	10·1
4	9·3	10·1	9·8
5	9·7	10·2	10·4
6	10·1	10·0	9·8
7	10·1	10·5	10·8
8	10·1	10·1	10·1
9	10·1	10·2	10·4
10	9·3	9·3	9·5
11	10·1	10·2	10·5
12	9·9	10·0	10·1

Interference—

Milner and Liederman[1] stated that the reaction was specific for the furfuraldehyde structure; methylfurfuraldehyde and hydroxymethylfurfuraldehyde reacted, but absorbed only slightly at 520 mμ. These workers found that formaldehyde, acetaldehyde, propionaldehyde and crotonaldehyde did not interfere, and we confirmed this.

IDENTIFICATION OF FURFURALDEHYDE

In the event of a prosecution, positive evidence of the identity of furfuraldehyde may be required. As the ultra-violet spectrum shows no fine structure, and infra-red analysis is impracticable because of the small amount of furfuraldehyde present in a sample of marked oil, a gas-chromatographic technique was evolved. The detection of furfuraldehyde in the sample proved to be impossible, owing to the complexity of the chromatogram, so an extraction method based on formation of the aldehyde - bisulphite compound was tried, as in Gent, Pomatti and Levin's method.[2]

Method

Apparatus—

Distillation assembly—Use an apparatus of the type recommended by the Institute of Petroleum[6] for distilling petroleum products boiling below 370° C (IP123/58).

Gas chromatograph—Fitted with an ionisation detector. Use a column containing 20 per cent. w/v of squalane on Celite 545 (100 to 120 mesh).

Reagents—

Diethyl ether, peroxide-free.
Sodium hydroxide solution, 10 per cent. w/v, aqueous.
Sodium bisulphite solution, 10 per cent. w/v, aqueous.
Sodium sulphate, anhydrous.

Procedure—

Concentrate the furfuraldehyde by separately collecting the first 60 ml of the 200 ml of distillate from the standard distillation carried out as recommended by the Institute of Petroleum (method C).[6] Transfer to a 100-ml separating funnel, and shake for 5 minutes with 10 ml of the 10 per cent. solution of sodium bisulphite. Allow the layers to separate, and run the lower aqueous layer containing the furfuraldehyde - bisulphite compound into a second 100-ml separating funnel. To this solution add 10 ml of the 10 per cent. solution of sodium hydroxide to liberate the furfuraldehyde, and shake for 2 minutes with 5 ml of peroxide-free diethyl ether. Allow to separate, and reject the lower layer. Dry the neck of the separating funnel, pour the ether extract through the neck of the funnel into a suitable stoppered receptacle, add a little anhydrous sodium sulphate, and set aside for about 15 minutes.

Inject about 50 μl (1 drop) of the anhydrous ether extract on to the top of the gas-chromatographic column, and record a chromatogram. Immediately after the chromatogram is complete, record a chromatogram for either pure furfuraldehyde or a suitable reference liquid whose retention time has been adopted as standard; in this laboratory, toluene is used as standard.

Suitable operating conditions when a Pye argon chromatograph is used are—

Column temperature—50° C.
Detector voltage—2000 volts for the sample; 1000 volts for the standard.
Amplifier sensitivity—× 10.
Argon input pressure—7 to 10 lb per sq. inch.
Chart speed—9 inches per hour.

Results

The retention time of furfuraldehyde on squalane at 50° C was found to be 1·06 relative to toluene (1·00), and, as these retention times were so close, it was considered desirable to ascertain whether or not the presence of toluene in gas oil could interfere with the identification of furfuraldehyde. Six samples of oil, treated as shown in Table II, were examined, with the results indicated.

Toluene, when present in these samples, gave a peak similar in size to that for furfuraldehyde, and this would represent a concentration of toluene in the sodium bisulphite extract of the order to be expected from the solubility of toluene in water. It was found that a second extraction with sodium bisulphite solution of the diluted ether extract was sufficient to remove any toluene present after the first extraction.

Discussion of the method

In order to detect the minimum concentration of furfuraldehyde in the ether extract, a large sample was injected, thereby deliberately overloading the column with ether. As the peak for ether emerges almost immediately and is widely separated from the peak for furfuraldehyde, the resultant broadening of the peak for ether does not cause interference. The concentration of furfuraldehyde in the ether solution was over thirty times that in the original sample of oil, and the minimum concentration detectable was found to be approximately 30 p.p.m. in the ether extract, corresponding to 1 p.p.m. in the original oil. It is therefore possible to identify furfuraldehyde in a mixture of one part of marked oil with nine parts of unmarked oil.

Peroxide-free diethyl ether is used to avoid any stray chromatographic peaks, which may occur with analytical-reagent grade ether. Extraneous peaks (notably for toluene) were observed on the chromatograms from time to time, probably caused by contamination of the glassware used in the extraction, which must be kept clean. Toluene is particularly objectionable, as its retention time under the conditions used is similar to that for furfuraldehyde and the peaks may overlap if toluene is present in large amount; it is, however, unlikely to be present in gas oil. Chromatograms have been recorded for n-butyraldehyde, n-hexaldehyde, n-heptaldehyde and 5-methylfurfuraldehyde, none of which gives a peak capable of interfering with the identification of furfuraldehyde.

Table II

Identification of furfuraldehyde in presence of toluene

Oil No.	Furfuraldehyde added, p.p.m.	Toluene added, %	Compound identified
9	5	3	Furfuraldehyde and toluene
12	Nil	Nil	Nil
12	Nil	3	Toluene
13	5	Nil	Furfuraldehyde
13	Nil	3	Toluene
13	5	3	Furfuraldehyde and toluene

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Methods for the Detection, Determination and Identification of Quinizarin in Hydrocarbon Oil

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A colorimetric method for detecting quinizarin and a spectrophotometric method for its identification and determination in concentrations from 0·2 to 2·0 p.p.m. in hydrocarbon oil are described.

In connection with the marking of heavy oils to distinguish between duty-paid oil for road vehicles (DERV) and duty-free oil for other purposes, quinizarin was chosen as one of the markers, as it fulfilled the necessary conditions stated by Harrison, Palframan and Rose in their paper on furfuraldehyde.[1]

DETECTION OF QUINIZARIN

Quinizarin is 1,4-dihydroxyanthraquinone; a search of the literature[2,3,4] produced the information that it is soluble in ethanol, ether, benzene, alkali or sulphuric acid and gives a blue colour with aqueous solutions of alkali, alkali carbonate or ammonia, but no method of determination was found.

A rapid test for quinizarin in solution can be carried out by dipping the end of a chromatographic column of alumina into the solution. Quinizarin is adsorbed in a band well defined by the formation of a red lake. With a solution in gas oil, however, the combined effects of the colour of the oil and the low concentration of quinizarin made this technique of little value.

The method described below for the roadside detection of quinizarin depends on the colour produced in alkaline solution. When a gas oil containing 2 p.p.m. of quinizarin is shaken with an aqueous solution of sodium hydroxide of any concentration between 1 and 30 per cent. w/v, quinizarin is extracted from the oil and produces a blue-violet colour in the aqueous phase. Stability of the colour increases with increasing concentration of sodium hydroxide, and is sufficient for a visual test for up to 30 minutes when a 1 per cent. w/v solution of alkali is used.

Method

Reagent—

Sodium hydroxide solution, 1 per cent. w/v, aqueous.

Procedure—

Pour 15 ml of the gas oil into a stoppered test-tube (6 inches × 0·75 inch), and shake for approximately 15 seconds with 2 ml of the sodium hydroxide solution. Allow the dispersion to separate into two layers (this may take 2 or 3 minutes), and observe the colour of the lower aqueous phase; a blue-violet colour indicates the presence of quinizarin. The test works well for the chosen concentration of marker (2 p.p.m.) and for a dilution of one part of marked oil with one part of unmarked oil. For a dilution of one part of marked oil with four parts of unmarked oil, the colour is not easy to see in a pale oil and impossible to see in a dark oil. The sensitivity of this test can be increased by using a larger volume of oil, but the same volume of sodium hydroxide solution.

DETERMINATION OF QUINIZARIN

An attempt was made to utilise the blue-violet colour produced with alkali as the basis of a quantitative spectrophotometric method, but several difficulties were encountered. Straightforward extraction of quinizarin from a marked gas oil by an aqueous solution of sodium hydroxide, with n-butyl alcohol to prevent emulsification, resulted in a solution that was not optically clear, owing to entrained droplets of oil. Acidification of this extract, subsequent extraction with diethyl ether, chloroform or light petroleum and then re-extraction

from the organic solvent with aqueous sodium hydroxide did not solve the problem. Centrifugation of the separated aqueous layer gave an optically clear solution, which, when spectrophotometrically examined, was found to have an absorption maximum at 560 mμ, but the colour was not completely stable; further, different gas oils gave different colours varying from blue to blue-violet.

During work on the problem of positive identification, the ultra-violet - visible spectrum of a 0·001 per cent. w/v solution of quinizarin in cyclohexane was plotted with an automatic recording spectrophotometer. A characteristic curve was produced having five peaks at 463, 476, 487, 508 and 521 mμ; those peaks at 508 and 521 mμ were extremely well defined. It was found that this method could be applied to the determination of quinizarin in marked samples of gas oil, thereby avoiding the difficulties mentioned above and giving results of greater accuracy than those obtained when the coloured aqueous solution was examined spectrophotometrically.

Method

Reagents—

Sodium hydroxide solution, 5 per cent. w/v, aqueous.
Hydrochloric acid, concentrated.
Cyclohexane, spectroscopically pure.
n-Butyl alcohol.

Procedure—

Shake 50·0 ml of the gas oil suspected to contain quinizarin with 5 ml each of the sodium hydroxide solution and n-butyl alcohol in a 100-ml separating funnel for 45 seconds. When the two layers have separated (this may take several minutes), run the aqueous phase into a second 100-ml separating funnel, and wash it through with about 2 ml of distilled water. Extract the gas oil with a further 5 ml of the sodium hydroxide solution by shaking for 45 seconds, allow the layers to separate, run the aqueous layer into the second separating funnel, and wash through with water as before. Repeat with a further 5 ml of the sodium hydroxide solution, and, after separation, add the aqueous layer to the previous extracts. Quinizarin forms a blue-violet colour with sodium hydroxide, and the third extract should be colourless; if it is not, extract with further 5-ml portions of the sodium hydroxide solution until a colourless extract is obtained. Acidify the combined extracts by adding 1 ml of concentrated hydrochloric acid for each 5 ml of sodium hydroxide solution used. When the solution has cooled, add, by pipette, 10·0 ml of spectroscopically pure cyclohexane, and shake for 30 seconds. After separation of the two layers, run the aqueous layer to waste, and use a clean dry pipette to transfer the amber-coloured cyclohexane solution to a stoppered parallel-sided 1-cm cell of an Optica automatic recording spectrophotometer. With pure cyclohexane as blank, plot the absorption spectrum from 420 to 540 mμ; use the peak at 521 mμ for determining quinizarin, as this is the most pronounced peak and is also furthest away from any possible interference from components of the gas oil. Prepare a standard curve by using a 0·001 per cent. w/v solution of recrystallised quinizarin in spectroscopically pure cyclohexane.

Discussion of the Method

Complete absorption due to components of the gas oil carried over in the separations usually occurs at wavelengths shorter than 420 mμ, but there is no interference with the five peaks produced by quinizarin. Since the quinizarin used to mark gas oils is of the quality normally available on the market and not specially purified, a more realistic result is obtained if an average absorption figure is taken as standard. Accordingly, 0·01 per cent. w/v solutions of quinizarin from different sources were prepared in cyclohexane and used to mark a gas oil at the level of 2·0 p.p.m. The 0·01 per cent. w/v solutions of quinizarin were also diluted to 0·001 per cent. w/v, and these solutions were used directly in the spectrophotometer. The aliquots of marked gas oil were extracted by the proposed procedure, and the optical densities of the cyclohexane extracts at 521 mμ were compared with those of the 0·001 per cent. w/v solutions of quinizarin; the results were—

Quinizarin sample No.	1	2	3	4
Optical density of 0·001% w/v solution in cyclohexane ..	0·244	0·240	0·235	0·253
Optical density of quinizarin extracted from gas oil ..	0·243	0·240	0·235	0·253

from which it can be seen that the extraction process is satisfactory.

The mean optical density of the 0·001 per cent. w/v solutions of quinizarin is 0·243, and there is a linear relationship between optical density and concentration of quinizarin.

In Table I are listed the results obtained when the proposed method was applied to different oils marked at the level of 2·0 p.p.m. of quinizarin; the mean figure of 0·243 for the optical density at 521 mμ was taken as being equivalent to 2·0 p.p.m. of quinizarin. A pale oil has a colour equal to or less than NPA 1, and a dark oil has a colour equivalent to NPA 3.[5]

TABLE I

RECOVERY OF 2 p.p.m. OF QUINIZARIN FROM VARIOUS OILS

Oil No.	Colour of oil	Optical density at 521 mμ	Quinizarin content found, p.p.m.
1	Pale	0·237	1·95
2	Dark	0·253	2·08
3	Dark	0·258	2·12
4	Pale	0·237	1·95
5	Pale	0·237	1·95
6	Dark	0·248	2·04
7	Dark	0·253	2·08
8	Dark	0·253	2·08
9	Dark	0·257	2·11
10	Dark	0·257	2·11
11	Pale	0·237	1·95

DISCUSSION OF RESULTS

The repeatability of the method is within 1 per cent. The result of an extraction from a pale oil is within 2 per cent. of the correct amount, but the result of an extraction from a dark oil may be high by up to 8 per cent. This was found to be caused by absorption from components of the gas oil carried over in the separations. All gas oils absorb slightly in the range 460 to 540 mμ, and the darker the oil, the greater is the absorption. In the laboratory, where the unmarked oils were available for blank determinations, a correction could be applied, and this brought all the results to within 2 per cent. of the correct amounts. However, in practice, unmarked oil is not available, and no method of producing a satisfactory blank from the marked oil has been found. When this is taken into account, together with the fact that samples of quinizarin from all the manufacturers in the United Kingdom varied in purity by 4 per cent. from the mean value, the proposed method will in the worst circumstances give a result within 10 per cent. of the amount present; for a pale-coloured oil, the result will be well within 5 per cent. of the amount present.

During the extraction, the colour in the sodium hydroxide solution may change from blue-violet to blue or fade completely, but this does not affect the determination. The method is satisfactory for concentrations of from 0·2 to 2·0 p.p.m. of quinizarin in gas oil and therefore can be used for a mixture of one part of marked oil diluted with nine parts of unmarked oil. The stability of quinizarin at a concentration of 2 p.p.m. in gas oil is good; determinations at the time of preparation of a dilution and 3 months later gave identical results.

IDENTIFICATION OF QUINIZARIN

The five peaks at 463, 476, 487, 508 and 521 mμ produced by the spectrophotometric analysis are used to identify quinizarin. Other dihydroxyanthraquinones absorb in this region, but they can be easily distinguished from one another. The 1,2- and 1,5- isomers have only two peaks in this region, and these occur between 400 and 440 mμ. The absorption spectrum of the 1,8- isomer is similar to that of quinizarin; it has five peaks, each occurring at a wavelength about 50 mμ shorter than the corresponding peak for quinizarin, but the peaks near 508 and 521 mμ are not at all well pronounced, as they are for quinizarin. Meek and Watson[3] reported on the spectrophotometric curves of six other polyhydroxyanthraquinones, none of which can be confused with that of quinizarin. It is possible to see the typical structure of the spectrophotometric curve even when the extraction is made from a gas oil containing 0·2 p.p.m. of quinizarin, *i.e.*, a dilution of one part of marked oil with nine parts of unmarked oil.

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Determination of Diquat Residues in Potato Tubers

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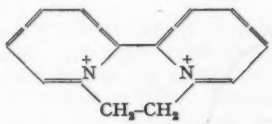
A specific method is described for determining diquat (1,1′-ethylene-2,2′-bipyridylium dibromide monohydrate) in potato tubers with a sensitivity of 0·01 p.p.m. It depends on measurement of the light absorption of reduced solutions of diquat after concentration and purification by cation-exchange chromatography. Correction for extraneous background absorption from natually occurring plant substances is made by using a "base line" method of calculation. The method has proved extremely reliable for determining traces of diquat in potato tubers harvested from crops sprayed in the field.

Diquat, 1,1′-ethylene-2,2′-bipyridylium dibromide monohydrate (**I**), formed by quaternising 2,2′-bipyridyl with ethylene dibromide, is the active ingredient of Reglone, a new herbicide and desiccant[1,2] recommended in the United Kingdom for destroying potato haulm. It is one of a group of quaternary bipyridylium salts whose herbicidal activity depends on their reduction in photosynthesising plant tissue.[3]



(I)

After application of diquat to the foliage of potato plants, death of the leaves is rapid, and the plant is completely desiccated when the crop is harvested 10 to 14 days later. Although diquat is known to be translocated within the plant, initial experiments indicated that only minute amounts of the compound were transported to the tubers. However, for the purpose of assessing possible hazards to the consumer, it was essential to confirm this by precisely determining concentrations present in the tubers after recommended field treatments. It was therefore essential to develop a reliable, sensitive and specific method for determining the compound.

Diquat carries two positive charges, is highly polar and cannot be extracted into water-immiscible organic solvents. However, owing to its cationic nature, it is retained on a cation-exchange resin when a dilute aqueous solution is allowed to percolate through the resin. The diquat can be displaced from the resin by a high concentration of an inorganic cation (*e.g.*, H^+, Na^+ or Ca^{2+}) and collected in a much smaller volume of solution.

Diquat in solution is easily reduced[4] with sodium dithionite, by transfer of one electron, to give a water-soluble and relatively stable free radical having an intense green colour.

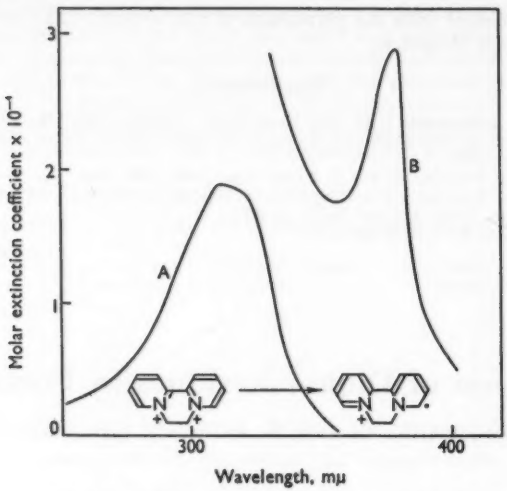


Fig. 1. Absorption spectra of an aqueous solution of diquat: curve A, before reduction; curve B, after reduction

Solutions of the free radical exhibit a sharp absorption peak at 378 mμ (see Fig. 1); this peak is of a greater intensity ($\epsilon = 28{,}000$) than that of unreduced diquat at about 310 mμ ($\epsilon = 19{,}200$), and spectroscopic measurement of reduced solutions provides a sensitive method for determining the compound.

When potato-tuber extracts are subjected to cation-exchange chromatography under the conditions necessary for concentrating diquat, the resulting effluents invariably contain small amounts of cationic plant constituents. These also exhibit ultra-violet absorption

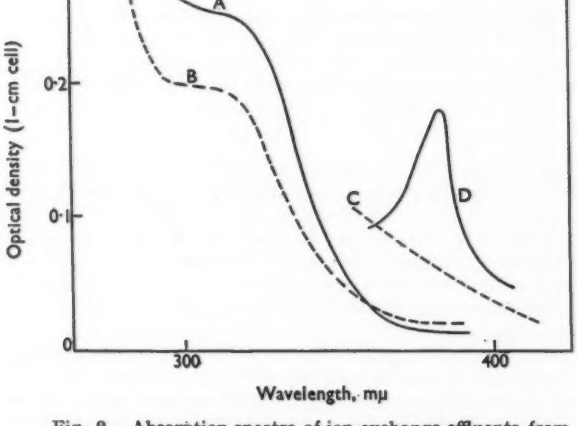


Fig. 2. Absorption spectra of ion-exchange effluents from hydrolysates of 1 kg of potato tubers. Broken-line curves are for tubers containing no diquat and full-line curves for tubers containing 0·4 p.p.m. of added diquat: curves A and B, before reduction; curves C and D, after reduction

both before and after reduction, but the intensity of this absorption is relatively less and more linear in the region of 378 mμ than at 310 mμ (see Fig. 2). For this reason, as well as for the additional sensitivity attained, it is more advantageous to determine diquat in such a solution by making measurements at the longer wavelength after reduction. Correction for the small amount of absorption from plant constituents can then be mathematically applied by using the "base-line" method of calculation first introduced[5] for the analysis of vitamin A. This procedure has the advantage of obviating the need for analysing control samples from untreated plants.

Other methods of determining diquat, *e.g.*, by spectrofluorimetry,[6] are inapplicable when small concentrations of the compound are to be determined in potato-tuber extracts, owing to interference from naturally occurring cations. The reduction method has the additional advantage of complete specificity for the herbicide.

Experimental

Extraction of diquat from potato tubers—

Diquat is highly soluble in water and is adsorbed from aqueous solutions on to many materials, including starch, activated carbon and soils. It seemed likely, therefore, that any diquat translocated to the tubers would be adsorbed on the starch or might even be occluded within the granules while deposition of the starch was proceeding. It was therefore clear that hydrolysis of the starch would be necessary in order to free any adsorbed diquat residues before the determination could be attempted, and this was confirmed by experiment.

Hydrolysis of a potato-tuber macerate by N sulphuric acid showed that hydrolysis of starch, as measured by the increase in concentration of reducing sugar,[7] was substantially complete after boiling for 1¼ hours. Hydrolysis of cell-wall polysaccharides probably continued beyond this stage, and there was a gradual increase in the ease of filtration of the hydrolysate after more prolonged boiling. A 5-hour period of boiling had the advantage of completely hydrolysing hemicellulosic material, leaving a filterable residue and ensuring maximum release of any diquat residues. There was no detectable loss of diquat in N sulphuric acid heated under reflux for this length of time.

Concentration of diquat on cation-exchange resin—

The acid hydrolysate of potato tubers is first neutralised with calcium carbonate to remove the large excess of hydrogen ions from solution before passage through the ion-exchange resin. Ethylenediaminetetra-acetic acid (EDTA) is also added in order to remove competing metallic ions that have high affinity for the resin, and the solution is made slightly alkaline. This procedure was found to result in a much decreased background light absorption in the final effluent containing diquat.

Zeo-Karb 225 (containing 8 per cent. of divinylbenzene) was the best of the complete range of Permutit cation-exchange resins examined. Diquat could be eluted from this resin by a small volume of solution containing a high concentration of H^+ (*e.g.*, 5 N hydrochloric acid) or other cations, such as Na^+ or Ca^{2+}. Reduction of diquat by sodium dithionite was not possible in acid or in the presence of Ca^{2+} ions, but proceeded satisfactorily in a saturated (approximately 6 M) solution of sodium chloride, which was found to be an efficient eluting agent for diquat.

Recovery of diquat from the resin is reproducible, although not quantitative. Complete recovery can be attained only by collecting a relatively large volume of effluent, but, by limiting the volume to 25 ml the lower recovery (approximately 80 per cent.) is offset by the gain in sensitivity over that which would be obtained if a larger volume of effluent were collected. Intermediate washing of the resin with dilute acid before the final elution with sodium chloride solution was found to remove a large proportion of the interfering plant material without eluting the diquat, thereby considerably increasing the sensitivity of the method.

When diquat is adsorbed on the resin from pure solution, the recovery under these conditions is 80 to 90 per cent. When diquat is added to a clear filtered potato-tuber hydrolysate, which is then allowed to pass through the resin, the recovery in the sodium chloride effluent is 60 to 70 per cent. This suggests that further loss of diquat occurs owing to its non-quantitative retention by the resin in the presence of a relatively large volume (1 litre) of potato-tuber hydrolysate. The cationic naturally occurring plant substances possibly compete with diquat cations for negative sites on the resin.

REDUCTION AND DETERMINATION—

Reduction of diquat by sodium dithionite was found to take place as effectively in 6 M sodium chloride as in water, but maximum absorption was at 379 instead of 378 mμ. The light absorption of the potato-tuber constituents eluted by 6 M sodium chloride is much less in the region of 379 mμ than at 310 mμ (where unreduced diquat has its absorption peak). The difference in sensitivity is clearly demonstrated in Fig. 2, which shows the absorption curves of the same mixtures before and after reduction. When sodium chloride effluents were reduced, it was necessary to neutralise the small concentration of acid present by using excess of alkali in the sodium dithionite reagent solution, and, after investigating different relative concentrations of reagents, initial difficulties experienced with the instability of the reduced product were overcome by using a 0·1 per cent. w/v solution of sodium dithionite in 3 N sodium hydroxide. Under these conditions, reduced diquat is stable for a period sufficient to permit accurate measurements of absorption.

Solid sodium dithionite can be kept under absolutely dry conditions without change, but in slightly moist air it is rapidly oxidised. When stored in a closed bottle for several months, it gradually loses its reducing power, and it was found to be necessary to use frequent supplies of fresh material and also to reduce standard solutions of diquat with each experiment.

As well as being oxidised by atmospheric oxygen, aqueous solutions of sodium dithionite slowly decompose to form thiosulphate and bisulphite. Decomposition is retarded in alkaline solution, and, under the conditions selected for the determination (*i.e.*, reduction of 10 ml of the diquat solution by 2·0 ml of a 0·1 per cent. w/v solution of sodium dithionite in 3 N sodium hydroxide, the excess of dithionite present was sufficient to continue reduction of a solution containing 5 μg of diquat per ml for up to 3½ hours, after which the concentration of dithionite was too low to effect complete reduction. It was therefore considered advisable to carry out reductions within 1 to 2 hours of preparing the alkaline dithionite reagent solution.

We found it necessary to correct for the slight absorption at 379 mμ contributed by both sodium chloride and alkaline dithionite solutions, and this was done by measuring the solution of reduced diquat against a blank mixture containing these reagents placed in the reference cell. The product of reduction of diquat in 6 M sodium chloride was stable for 1 hour when measured against an alkaline dithionite - 6 M sodium chloride blank prepared at the same time. However, small errors were incurred if the reduced diquat was measured against a fresh blank or a blank prepared a long time previously, as the diluted dithionite solution remained stable for only about 20 minutes. For this reason, it was found advisable to prepare a fresh blank at the same time as each diquat solution and to take readings 1 minute after mixing the solutions (or as soon after as possible) in order to minimise any slight errors due to different rates of decomposition of the dithionite in the blank and diquat solutions.

When 10-ml aliquots of solutions of diquat in water or 6 M sodium chloride were reduced by 2·0-ml portions of a 0·1 per cent. w/v solution of sodium dithionite in 3 N sodium hydroxide and the optical densities at 378 or 379 mμ of the resulting solutions were measured in 1-cm cells against similar blank solutions after 1 minute, the relationship between optical density and concentration was linear over the range 1 to 5 μg of diquat per ml. Beer's law was obeyed, but the gradient of the line varied slightly on different occasions, owing to changes in the reducing power of the dithionite. For concentrations of diquat below 1 μg per ml, greater sensitivity was attained by using 4-cm cells. Under these conditions, the relationship between optical density and concentration was linear down to 0·2 μg of diquat per ml, and a concentration of 0·1 μg per ml was just detectable. With a 4-cm light path, a concentration of 1 μg of diquat per ml gave, after reduction, an optical density of approximately 0·32.

CORRECTION FOR BACKGROUND ABSORPTION

The optical densities at 379 mμ of ion-exchange effluents from control (untreated) potato tubers increased on reduction with the dithionite reagent solution, often to the extent of about 50 per cent. Hence, certain naturally occurring plant substances, which are concentrated on the resin and eluted with the diquat, are reducible to give a product or products whose absorption at this wavelength is greater than that of the unreduced forms. In mixtures containing diquat it is therefore impossible to ascertain the contribution of the plant material by measuring the optical density at 379 mμ before and after addition of the dithionite solution, in spite of the fact that unreduced diquat has zero absorption at this wavelength.

Equations were derived for calculating the absorption at 379 mμ contributed by reduced diquat present in ion-exchange effluents also containing plant substances that contribute

background absorption to an unknown and variable extent. This method is based on that described[5] for determining vitamin A in mixtures, assuming that the background absorption is linear in the narrow wave-band chosen, and it entails accurate measurement of the intensity of absorption at three wavelengths. From these values are calculated the contribution of the pure compound in the mixture (after having measured the relative optical densities of the compound alone at these wavelengths).

The background absorption of reduced effluents from potato tubers was found to be sufficiently linear in the range 375 to 385 mμ to permit application of this method, and equations were derived in two ways to correct the composite absorption for the unknown amount of background material mixed with the reduced diquat. For equation (1), the wavelength of maximum absorption for reduced diquat (379 mμ) was selected, together with supplementary wavelengths (375 and 385 mμ) on either side of the absorption peak. For equation (2) were used 379 mμ and supplementary wavelengths of 375 and 383 mμ, at which the optical densities of reduced diquat in sodium chloride solution were found to be the same. The necessary constants for the optical-density ratios of pure diquat were determined by reducing solutions of diquat in 6 M sodium chloride (10 ml) with a 0·1 per cent. w/v solution of dithionite in 3 N sodium hydroxide (2 ml). The optical densities at the four wavelengths mentioned above were accurately measured, and, from an average of ten determinations, it was found that—

$$K_1 = \frac{E_{379}}{E_{375}} = 1{\cdot}260$$

$$K_2 = \frac{E_{379}}{E_{385}} = 1{\cdot}535$$

$$K_3 = \frac{E_{375} \text{ or } E_{383}}{E_{379}} = 0{\cdot}799 = \frac{1}{K_1}$$

DERIVATION OF EQUATION (1)—

If E_{379}, E_{375} and E_{385} are the observed optical densities of the mixture at 379, 375 and 385 mμ, respectively, and b_{379}, b_{375} and b_{385} are the background contributions to these figures, then $(E_{379} - b_{379})$, $(E_{375} - b_{375})$ and $(E_{385} - b_{385})$ will be the contributions of diquat to the observed absorptions at these wavelengths. Since these contributions are for diquat only, then—

$$\frac{E_{379} - b_{379}}{E_{375} - b_{375}} = K_1 = 1{\cdot}260 \quad .. \quad .. \quad .. \quad .. \quad (i)$$

$$\frac{E_{379} - b_{379}}{E_{385} - b_{385}} = K_2 = 1{\cdot}535 \quad .. \quad .. \quad .. \quad .. \quad (ii)$$

Since the background absorption is linear—

$$b_{379} = 379\,m + c$$
$$b_{375} = 375\,m + c$$
$$b_{385} = 385\,m + c$$

where m and c are constants for the straight line.

By eliminating the constant c from these expressions—

$$b_{375} = b_{379} - m\,(379 - 375)$$
$$b_{385} = b_{379} - m\,(379 - 385)$$

Substitution of these values for b_{375} and b_{385} in equations (*i*) and (*ii*) and solving for b_{379} gives—

$$b_{379} = 2{\cdot}28\,E_{375} + 1{\cdot}52\,E_{385} - 2{\cdot}79\,E_{379}.$$

The corrected value for the absorption of diquat at 379 mμ is given by—

$$E_{379}\,(\text{corrected}) = E_{379} - b_{379}$$

and therefore

$$E_{379}\,(\text{corrected}) = 3{\cdot}79\,E_{379} - 2{\cdot}28\,E_{375} - 1{\cdot}52\,E_{385} \quad .. \quad .. \quad .. \quad (1)$$

DERIVATION OF EQUATION (2)—

If E_{375} and E_{383} are the observed optical densities of the mixture at 375 and 383 mμ, at which wavelengths the absorptions of reduced pure diquat in 6 M sodium chloride are equal, then, from the similar triangles GHK and FHJ in Fig. 3—

$$\frac{KH}{JH} = \frac{GK}{FJ} = \frac{GK}{AL}$$

GK is therefore equal to $AL.\frac{KH}{JH}$

$$= (E_{375} - E_{383}) \left(\frac{383 - 379}{383 - 375}\right) = \tfrac{1}{2}(E_{375} - E_{383})$$

Now the absorption contributed to the mixture by diquat is given by $(E_{383} - HN)$ at 383 mμ and $(E_{379} - GM)$ at 379 mμ, *i.e.*—

$$K_3 = 0{\cdot}799 = \frac{E_{383} - KM}{E_{379} - KM - GK} = \frac{E_{383} - KM}{E_{379} - KM - \frac{1}{2}(E_{375} - E_{383})}$$

from which—

$$KM = 2{\cdot}985\, E_{383} - 3{\cdot}973\, E_{379} + 1{\cdot}986\, E_{375}$$

But—

$$E_{379}\,(\text{corrected}) = E_{379} - KM - GK$$

and substitution for KM and GK gives—

$$E_{379}\,(\text{corrected}) = 4{\cdot}97\, E_{379} - 2{\cdot}49\,(E_{375} + E_{383}) \quad .. \quad .. \quad .. \quad (2)$$

Since differences between observed optical densities are involved in these equations, it is clear that the precision of measurement must be greater than that required in the final result. Measurements are made at wavelengths where the absorption curve falls steeply,

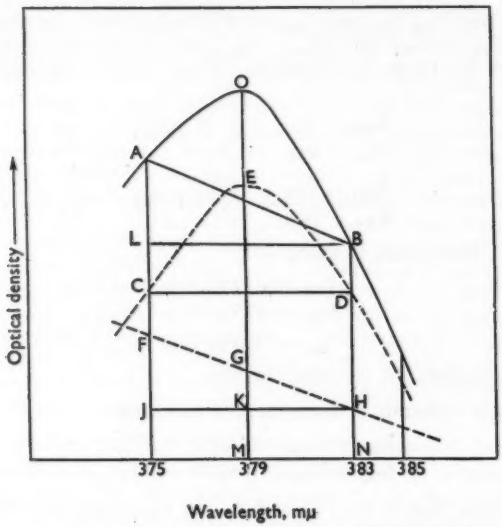


Fig. 3. Absorption spectrum of reduced diquat in ion-exchange effluent. Curve AOB represents the observed results and is a summation of the curves for the absorptions of reduced diquat (CED) and background material (FGH), both of which are unknown. The absorption of reduced diquat is the same at 375 and 383 mμ, *i.e.*, line CD is horizontal, and line AB therefore represents the gradient of the background absorption

and it was considered advisable to re-adjust the wavelength settings between determinations on duplicate samples. This, in conjunction with the use of equations (1) and (2), gives four results for each sample, and these results serve as a check on each other and on any errors in measurement. Excellent replication has been obtained between duplicate observed optical densities when using this method, and the agreement between the corrected absorptions calculated from the two equations is usually within 10 per cent.

Obviously, the limiting factor in the accuracy of the method is the degree of linearity of the background absorption. This has been examined for numerous samples of potato tubers of Majestic, King Edward and Redskin varieties, and for all samples the absorption was nearly linear in the range 375 to 385 mμ under the conditions described for the determination. Any significant deviations from linearity should be detected immediately by increased differences between the results calculated from the two equations. If the difference is large, it is advisable to use control samples of plant extract alone in order to detect any serious deviations from linearity, since, in this event, the equations would be invalid. In fact, when the equations were applied to effluents from untreated tubers, spurious low results were found in a few instances, corresponding to apparent-diquat contents of less than 0·01 p.p.m. in the tubers.

The accuracy of results calculated by using equations (1) and (2) has been confirmed by (*a*) applying the equations to solutions of pure diquat in 6 M sodium chloride, which gave results of 100 per cent., and (*b*) adding known amounts of diquat to ion-exchange effluents containing plant material from untreated tubers, which gave results of 92 to 100 per cent.

The method of calculation described has the particular advantage of rendering unnecessary the analysis of control samples from untreated tubers. Not only does this decrease the amount of labour required, but it also eliminates substantial errors, since variation in the chemical composition of biological samples is such that control and treated samples rarely have exactly the same content of interfering material. It also provides a method directly applicable to commercial samples when no controls are available.

Method

Diquat is extracted from potato tubers by boiling them with dilute sulphuric acid, and the extract is neutralised and passed through a column of cation-exchange resin. Diquat is retained on the resin and finally eluted by a small volume of sodium chloride solution. It is determined by measuring the optical density of the reduced solution in the region of 379 mμ, a correction being made for the irrelevant background absorption.

Apparatus—

Macerator—A Top-Drive macerator obtained from Townson and Mercer Ltd, was used.

Boiling flasks—Flasks of capacity 2 litres fitted, by means of standard ground-glass joints, with reflux condensers.

Tubes for cation-exchange columns—Glass tubes, 40 to 50 cm long and 9 to 10 mm internal diameter, with taps (25-ml burettes are suitable). Automatic-feed or flow-control devices are advantageous.

Spectrophotometer—A Unicam SP500 or SP600.

Reagents—

Sulphuric acid, 18 N—Cautiously add, with stirring, 1 litre of concentrated sulphuric acid to about 1 litre of water, allow to cool, and dilute to 2 litres.

Hydrochloric acid, 2 N—Dilute 175 ml of concentrated hydrochloric acid to 1 litre.

Sodium chloride solution, approximately 6 M—Shake 400 g of analytical-reagent grade sodium chloride with a litre of water until the solution is saturated.

Ethylenediaminetetra-acetic acid, disodium salt.

Capryl alcohol.

Cation-exchange resin—Use Permutit Zeo-Karb 225 (52 to 100 mesh), containing 8 per cent. of divinylbenzene, in the sodium form. Pack the column by adding approximately 3 g of the resin to about 15 ml of water in the tube, so that the settled column, supported on a pad of glass-wool, is 5 cm in length. Pack a small pad of glass-wool above the resin, allow the water to run away, and pass successively through the column at 2 to 5 ml per minute 25 ml each of 6 M sodium chloride and water; the column is then ready for use. Keep the resin covered with water or solution at all times. Use a freshly prepared column of new resin for each test.

Sodium dithionite solution, 0·1 per cent. w/v, in 3 N sodium hydroxide—This reagent is unstable; it should be prepared immediately before use and on no account used after it has been prepared for longer than 1½ hours.

STANDARD SOLUTIONS OF DIQUAT—

Stock solution, 250 p.p.m.—Dissolve 25 mg of pure diquat in 6 M sodium chloride, and dilute to 100 ml with 6 M sodium chloride.

Solution A, 10 p.p.m.—Dilute 10 ml of stock solution to 250 ml with 6 M sodium chloride.

Solution B, 2·5 p.p.m.—Dilute 25 ml of solution A to 100 ml with 6 M sodium chloride.

Solution C, 1·5 p.p.m.—Dilute 15 ml of solution A to 100 ml with 6 M sodium chloride.

Solution D, 1·0 p.p.m.—Dilute 10 ml of solution A to 100 ml with 6 M sodium chloride.

Solution E, 0·5 p.p.m.—Dilute 5 ml of solution A to 100 ml with 6 M sodium chloride.

These solutions are stable under normal laboratory conditions, but must not be exposed to direct sunlight.

EXTRACTION AND CONCENTRATION OF DIQUAT—

Take about 2500 g of tubers at random from the sample provided, wash free from soil, and remove surplus water with a dry cloth. Cut each tuber into four approximately equal segments, and reject two opposite segments from each tuber. Cut the remaining segments into small pieces (approximately 1-cm cubes) with a chipping machine or knife, thoroughly mix the pieces, and weigh out a 500-g portion for the determination.

Place approximately half of the weighed portion into the macerator jar (since this will not accommodate the whole 500 g), add 150 ml of water and 5 ml of 18 N sulphuric acid, macerate for 3 minutes, and transfer the macerated material to a 2-litre boiling flask. Treat the remainder of the 500-g portion in the same way, and add the macerated material to the contents of the boiling flask. Rinse the macerator jar with 40 ml of water, transfer the rinsings to the 2-litre flask, and then add 34 ml of 18 N sulphuric acid and a few drops of capryl alcohol. Swirl the flask, support it on an asbestos-coated gauze over a tripod, attach a reflux condenser, and gradually heat with a bunsen burner until the contents of the flask boil gently. Swirl the flask occasionally to prevent local overheating and charring, and allow the solution to boil steadily. Boil under reflux for 5 hours, and allow to cool (the mixture can be left overnight at this stage).

Quantitatively transfer the contents of the flask to a 3-litre beaker, and neutralise the excess of acid by adding 50 g of powdered calcium carbonate in small portions; keep the suspension well stirred during the addition. (Add a further few drops of capryl alcohol during the addition of calcium carbonate to decrease the amount of froth formed.) Add 10 g of anhydrous sodium carbonate, again in small portions, and stir to dissolve. Pour the suspension on to a Whatman No. 5 filter-paper supported in a 16-cm Buchner funnel, apply suction, and collect the filtrate in a 2-litre filter flask. Suck the residue dry, and wash it successively with two 100-ml portions of water; allow the first portion to be sucked through completely before the second is added. Return the clear filtrate to the original beaker, add 5 g of EDTA, and stir to dissolve. With use of a pH meter, adjust the pH of the solution to between 7·1 and 7·5 by adding small amounts of anhydrous sodium carbonate (a total of about 1·5 g will be needed).

Quantitatively transfer the solution to a 1-litre separating funnel supported above the previously prepared cation-exchange column. Adjust the rate of flow to between 7 and 8 ml per minute, and allow all the solution to percolate through the column. Then, at the rate of about 2 ml per minute, allow 25 ml of water, 50 ml of 2 N hydrochloric acid and 25 ml of water (in that order) to percolate through the column. (The process can be discontinued overnight at this stage, provided that the resin is covered with a small volume of water.)

Run the water from the column, and elute the diquat by passing 25 ml of 6 M sodium chloride through the column at about 1 ml per minute. Collect the effluent in a 25-ml calibrated flask, and adjust the volume to 25 ml with effluent.

DETERMINATION OF DIQUAT—

By pipette, transfer a 10-ml aliquot of effluent to a 25-ml stoppered cylinder, add 2·0 ml of sodium dithionite solution, and mix. Within 15 minutes of adding the sodium dithionite, measure the optical densities of the solution at 375, 379, 383 and 385 mμ in 4-cm glass cells

with a spectrophotometer; use as reference solution a mixture of 10 ml of 6 M sodium chloride and 2·0 ml of sodium dithionite solution, prepared at the same time. Record the observed optical densities at these wavelengths (E_{375}, E_{379}, E_{383} and E_{385}).

Measure in a similar manner, concurrently with each series of determinations, the optical densities at 379 mμ of 10-ml aliquots of standard diquat solutions B, C, D and E, each reduced with 2·0 ml of sodium dithionite solution; use a reference solution prepared as indicated above. (Standard solutions containing higher concentrations of diquat must be prepared if the optical density recorded for the sample solution is greater than that of standard solution B). From these readings, construct a graph relating optical density at 379 mμ to concentration of diquat in parts per million.

CALCULATION—

For each solution tested, correct the optical density at 379 mμ (E_{379}) for irrelevant absorption by means of equations (1) and (2). Let the optical density corrected by equation (1) be E'_{379} and by equation (2) E''_{379}.

$$E'_{379} = 3{\cdot}79\,E_{379} - 2{\cdot}28\,E_{375} - 1{\cdot}52\,E_{385}$$

$$E''_{379} = 4{\cdot}97\,E_{379} - 2{\cdot}49\,(E_{375} + E_{383})$$

These equations are based on the assumption that the irrelevant absorption is linear, or nearly so, with respect to wavelength over the range 373 to 385 mμ. The optical density of reduced pure diquat in 6 M sodium chloride is the same at 375 mμ and 383 mμ, and the difference between the observed optical densities at these wavelengths, E_{375} and E_{383}, therefore indicates the gradient of the irrelevant absorption.

Let the mean of E'_{379} and E''_{379} be E_{379} (mean corrected), and, from the previously constructed calibration graph, ascertain the concentration of diquat present in the final effluent corresponding to this value. Let this concentration be Y p.p.m., and calculate the concentration of diquat in the sample (see Note) from the equation—

$$\text{Diquat content, p.p.m.} = \frac{\text{Volume of effluent, ml}}{\text{Weight of sample, g}} \times \frac{100}{\text{Recovery, \%}} \times Y$$

$$= \frac{25}{500} \times \frac{100}{\text{Recovery, \%}} \times Y$$

$$= \frac{5Y}{\text{Recovery, \%}}$$

NOTE—The amount of diquat present in the effluent does not represent the total amount in the sample taken for analysis, as there are losses at the various stages of the assay. The amount therefore has to be corrected by applying a factor dependent on the percentage recovery. Before the method is routinely applied, each operator must carry out a series of determinations on samples prepared by adding diquat at the appropriate concentration to unhydrolysed macerates of control potato tubers in order to ensure that a reproducible technique has been acquired. Also, during the routine analysis of samples, recovery experiments should be carried out in the same way on each of the varieties of potato being tested.

DISCUSSION OF RESULTS

Diquat is normally obtained pure as a crystalline pale-yellow monohydrate. It should be understood that the 1,1′-ethylene-2,2′-bipyridylium ion is determined, but concentrations are expressed in terms of the monohydrated dibromide.

LIMITS OF DETECTION—

The weight of potato-tuber sample taken for each individual determination is 500 g, and diquat can be detected in ion-exchange effluents at a minimum concentration of about 0·2 μg per ml. Twenty-five millilitres of effluent are collected, so that this minimum represents 5·0 μg of diquat, equivalent to a detection limit in the tubers of 0·01 p.p.m. Further sensitivity below this level is ultimately limited by the concentration of interfering cationic compounds present in the effluent.

RECOVERY—

In these laboratories, the average recovery in a series of experiments by two operators was 59 per cent. This is shown in Table I, which records recoveries of diquat added to chopped potato tubers from untreated plants before the determination.

TABLE I

RECOVERY OF DIQUAT ADDED TO 500-g PORTIONS OF UNTREATED POTATO TUBERS (MAJESTIC VARIETY)

Diquat added, p.p.m.	Diquat recovered by operator A				Diquat recovered by operator B			
	Equation (1) used		Equation (2) used		Equation (1) used		Equation (2) used	
	p.p.m.	%	p.p.m.	%	p.p.m.	%	p.p.m.	%
0·048	0·031	65	0·026	55	0·029	60	0·028	58
	—	—	—	—	0·026	54	0·025	52
	—	—	—	—	0·033	69	0·031	65
0·096	0·060	62	0·056	59	0·065	68	0·063	66
	0·052	54	0·049	52	0·054	56	0·048	50
	0·054	56	0·051	53	0·058	60	0·054	56
	0·051	54	0·045	47	—	—	—	—
	0·057	59	0·054	56	—	—	—	—
	0·054	56	0·052	54	—	—	—	—
	0·065	68	0·061	64	—	—	—	—
0·192	0·127	66	0·118	61	0·124	65	0·119	62
	—	—	—	—	0·120	62	0·111	58
	—	—	—	—	0·117	61	0·105	55
Average		60		56		62		58
Mean average recovery, %						59		

Statistical analyses of these results showed that (*a*) the mean difference between operators was not significant, (*b*) the difference between results obtained from the two equations was consistent, but small when compared with the difference between experiments and (*c*) the standard error of a single determination was ±5·3 per cent.

FIELD TRIALS—

During 1959, diquat was used for destroying potato haulm in extensive field trials at ten independent sites in the United Kingdom in order to assess the residual levels in the harvested tubers. In these experiments the rates of application per acre were from 1½ to 4 lb of diquat in 20 gallons of water. All treatments were replicated four times, and 10-lb samples of tubers were collected at random from each experimental plot. At least two of the four replicate samples were analysed by the proposed method, and recovery experiments were carried out concurrently on the varieties tested. Some of the results are summarised in Table II. It was found that the diquat contents of the tubers increased with the rate of application, although not in direct proportion. At the commercially recommended rates, *i.e.*, 1·5 to 2 lb of diquat per acre, the residues were less than 0·05 p.p.m. For control tubers, the apparent-diquat contents obtained by application of the two equations were of the order of 0·01 p.p.m. No correction for this was made in the results for the treated tubers, which were all less than 0·1 p.p.m. In the recovery experiments with 0·048 to 0·24 p.p.m. of added

TABLE II

DIQUAT RESIDUES FOUND IN POTATO TUBERS AFTER FIELD TRIALS IN 1959

The figures in brackets are the numbers of determinations made.
All results have been corrected for recovery of 59 per cent.

Variety of potato	Residue found after application of—			
	no diquat, p.p.m.	1·5 lb of diquat per acre, p.p.m.	2 lb of diquat per acre, p.p.m.	4 lb of diquat per acre, p.p.m.
Arran Pilot ..	0·015 (2)	0·027 (5)	0·025 (4)	—
Majestic	0·012 (3)	0·015 (3)	0·020 (2)	—
Redskin	0·008 (2)	0·023 (5)	0·034 (4)	0·091 (4)
Record	0·010 (2)	0·019 (5)	0·023 (4)	0·038 (4)
Over-all mean and standard error ..	0·011 ± 0·002 (9)	0·022 ± 0·002 (18)	0·026 ± 0·002 (14)	0·051 ± 0·014 (8)

diquat, the mean recovery (± the mean standard error) based on twenty-four determinations was 58 ± 2·2 per cent., which compared favourably with that shown in Table I. There appeared to be no significant difference in the recoveries obtained for different varieties of potatoes.

In 1960, further experiments on potatoes were carried out on 1-acre plots at twelve sites, with the object of comparing applications of 2 lb of diquat in small (20 gallons) and large (100 gallons) volumes of water per acre; results for diquat found in the tubers are shown in Table III. From these results it is clear that, at the recommended rate of application of diquat, there was no significant difference between the residues found in tubers sprayed with the solutions of small and large volume. Untreated controls had apparent-diquat contents of the order of 0·005 p.p.m., whereas treated tubers contained residues of about 0·04 p.p.m.

TABLE III

DIQUAT RESIDUES FOUND IN POTATO TUBERS AFTER APPLICATION OF 2 lb OF DIQUAT PER ACRE IN 1960 FIELD TRIALS

The figures in brackets are the numbers of determinations made.

All results have been corrected for recovery of 59 per cent.

Variety of potato	Residue found in control, p.p.m.	Residue found after application of diquat in—	
		20 gallons of water, p.p.m.	100 gallons of water, p.p.m.
King Edward	0·005 (4)	0·041 (7)	0·043 (9)
Majestic	0·004 (2)	0·039 (5)	0·037 (5)
Dunbar Standard	0·004 (2)	0·019 (2)	0·041 (2)
Redskin	0·005 (1)	0·036 (3)	0·033 (3)
Over-all mean and standard error	0·004 ± 0·001 (9)	0·037 ± 0·006 (17)	0·040 ± 0·005 (19)

METABOLISM—

In experiments with plants grown in boxes and sprayed with ^{14}C-labelled diquat it was shown that the total radioactivity in the tubers could be completely accounted for as unchanged diquat, residues of which were small (0·05 p.p.m.); the amount of any radioactive metabolite of diquat translocated to the tubers was therefore negligible. This evidence, together with the absence of detectable metabolic products of labelled diquat in the foliage, indicates that hazards to the consumer arising from the formation of toxic metabolites of diquat may be discounted.

APPLICATION OF METHOD TO OTHER CROPS—

With slight modifications to the procedure for extraction and processing, the method was found to be applicable to the determination of diquat in other crops, including ginned cotton seeds, onions and peas. Provided that the diquat can be successfully extracted from the crop, the main criterion of applicability of the method is linearity of the light-absorption curve in the region 375 to 385 mμ for reduced naturally occurring plant compounds in the ion-exchange effluent.

We thank Mr. J. H. Dunn for his part in the early stages of the development of the method and for helpful discussions. Dr. S. H. Crowdy, Mr. G. Douglas and Mr. M. G. Ashley planned the field trials, and Mr. Ashley supervised the subsequent analysis of the field samples and collated the results. The technical assistance of Mrs. Dorothy Pettifer is also gratefully acknowledged.

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A Rapid Method for Determining Indium by Neutron Activation

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A method is described for determining indium in complex mixtures by neutron-activation analysis. The indium is rapidly isolated from other active components by passage through a column consisting of dithizone and an organic solvent retained on cellulose acetate. By this means, the high potential sensitivity afforded by the measurement of the 54-minute ^{116}In nuclide can be conveniently exploited.

Neutron-activation analysis is an extremely sensitive method for the quantitative determination of many elements.[1] Nevertheless, the activity induced in a complex mixture during irradiation is likely to be due to a number of nuclides, and care must be taken to ensure that the radiation from the element to be determined can be distinguished from all other activity present. It is sometimes possible to carry out measurements without prior chemical separation by correcting for interfering radiation after analysis of the decay curve of the sample, by excluding unwanted radiation with use of gamma-ray spectrometry or by choosing irradiation and decay times so that, at the time of measurement, the activity of the sample is due solely to the element being determined. Usually, however, chemical separation is effected to achieve radiochemical purity, and a known amount of inactive carrier is first added to avoid microchemical manipulation and to permit assessment of the chemical yield. If this separation is lengthy and the half-life of the activity being measured is short by comparison, sensitive determination may be precluded. Further, the more complicated the separation technique, the greater will be the likelihood of low over-all chemical yield, and associated with this will be reduced sensitivity. It is therefore desirable that the method of separation should be as rapid and simple as possible.

A sensitive method for determining indium is by measuring the activity of the 54-minute ^{116}In nuclide, which could be expected to give an ultimate sensitivity of 5×10^{-12} g after irradiation for one half-life in a flux of 10^{12} neutrons per sq. cm per second (if an efficiency of counting of 10 per cent. and 10 counts per minute above background are assumed[2]). However, in view of the relatively short half-life of the isotope, samples must be rapidly processed if the high potential sensitivity of the method is to be exploited. The usual techniques for separating indium from complex mixtures, such as rocks or minerals, involve a number of precipitation and extraction steps,[2,3] but it was thought that the separation could be considerably simplified by using a column consisting of a solution of a chelating agent retained on a solid support.

Experimental

Column material—

Many chelating agents used extensively in two-phase systems for extracting metals are hydrophobic and cannot be used in solid form as column material, since the rate of formation of the metal complex in the absence of organic solvent is exceedingly slow. Nevertheless, solutions of these substances can be used as stationary phase for column operation if they are retained on a solid support that holds them firmly enough to prevent them from being stripped off the column by passage of an aqueous phase and yet does not impede reaction between metal and complexing agent. Silica gel has been used to retain solutions of dithizone in carbon tetrachloride or chloroform,[4,5] but, as careful pre-treatment is necessary to obtain a gel of satisfactory form and purity, the possibility of using other support materials was investigated. It was found that a number of polymers could be "gelled" sufficiently with some organic solvents to permit appreciable retention of the solvent without causing the separate particles to coalesce. The best of the polymers investigated, cellulose acetate, retained dithizone dissolved in a mixture of chloroform and carbon tetrachloride so firmly

that the support could be made into a slurry with an aqueous phase or mechanically packed into a column without stripping. Only when high pressures were used to increase the rate of flow did the organic solution leave the support.

The ability of commercially available cellulose acetate to retain chloroform - carbon tetrachloride was found to vary considerably from sample to sample. Some cellulose acetates were capable of holding little organic solvent, which resulted in decreased extraction of the metal and poor separation factors, even under optimum conditions; the weight of organic solvent on a satisfactory support was at least three times the original weight of the cellulose acetate.

Studies with indium tracer indicated that the element could be quantitatively extracted from a variety of aqueous solutions buffered to a pH of approximately 5 if the indium solution was passed down a dithizone column of the type discussed above. The complex formed was sufficiently stable to permit the column to be washed with a number of aqueous phases of different compositions without dissociating the indium dithizonate, but N hydrochloric acid eluted the indium quantitatively.

ACTIVATION OF SAMPLES CONTAINING INDIUM—

The possible errors and limitations of neutron-activation analysis for determining indium have been discussed elsewhere[2] and will not be reconsidered here. The effectiveness of the separation of indium in a state of radiochemical purity from complex mixtures (a number of rocks and a meteorite) by means of a dithizone column was investigated. The purity of the indium activity isolated from the irradiated samples was assessed by comparing the gamma-ray spectrograms and the decay curves with those of standards prepared from irradiated pure indium foil. The samples of rock used were a fayalite ferrogabro from the Skaergaard Intrusion, East Greenland (specimen collection No. E.G. 4327), the standard diabase W-1 from Centerville, Virginia, and the standard granite G-1 from Westerly, Rhode Island; the meteorite was the coarse octahedrite Canyon Diablo.

IRRADIATION—

About 100 mg of meteorite drillings or powdered rock were accurately weighed and sealed into silica tubes (4 mm internal diameter). A standard solution of indium in dilute nitric acid was prepared from pure indium foil, and portions of this solution, each containing about 10 μg of indium, were also weighed and sealed into similar silica tubes. Samples and standards were then packed together and activated in a flux of approximately 10^{12} neutrons per sq. cm per second for 1 hour. Analysis of the samples of meteorite and rock was begun 5 and 15 minutes, respectively, after their removal from the reactor.

PREPARATION OF SUPPORT—

Purified dithizone[6] was dissolved in a mixture of equal volumes of chloroform and carbon tetrachloride to give a solution that was almost saturated. Cellulose acetate (16- to 22-mesh, in flake form) was placed in a beaker and stirred as dithizone solution was added to it until a little free liquid appeared at the bottom of the beaker. Surplus solvent was removed by stirring the support in a stream of air until a free-running powder was obtained, and this was then made into a slurry with a 2 N sodium acetate - hydrochloric acid buffer solution (pH 5) that had previously been equilibrated with chloroform. The slurry was poured into a chromatographic tube of the normal type, and the cellulose acetate was held in position by placing a sintered-glass disc on top of the column.

The satisfactory functioning of a column material of this type is dependent on the retention of an appreciable amount of organic solvent by the cellulose acetate, and great care must therefore be exercised not to over-dry the support before preparing the slurry. The columns used were approximately 2 cm in diameter and 20 cm in depth; rates of flow were normally 15 to 25 ml per minute.

SOLUTION OF SAMPLES—

Samples of rock were dissolved after being either heated to fumes with a mixture of hydrofluoric, perchloric and nitric acids or sintered with sodium peroxide. With both procedures, it was necessary to precipitate and remove all silica before passing the solution through the column; this avoided separation of silica during passage down the column, which could decrease or even prevent flow of liquid. The more complicated method of solution, involving sintering with peroxide, is described under "Procedure."

Samples of the meteorite were easily dissolved in a mixture of hydrochloric and nitric acids.

Method

Reagents—

Indium carrier solution—Prepare a solution containing 4 mg of indium per ml in dilute nitric acid.

Sodium peroxide, powdered.

Ammonia solution, sp.gr. 0·880.

Perchloric acid, 72 per cent.

Sodium acetate, 2 N.

Acetate buffer solution—Adjust the pH of 2 N sodium acetate to 5 by adding hydrochloric acid, sp.gr. 1·18, and saturate with chloroform.

Hydrochloric acid, sp.gr. 1·18.

EDTA solution—Prepare a 5 per cent. w/v solution of the disodium salt of ethylenediaminetetra-acetic acid in water, and saturate with chloroform.

Perchloric acid solution, dilute—Saturate 0·01 N perchloric acid with chloroform.

Hydrochloric acid solution—Saturate aqueous N hydrochloric acid with chloroform.

Oxine solution, 5 per cent. w/v, in 96 per cent. ethanol.

Ammonium acetate solution, 2 per cent. w/v, aqueous.

Procedure—

Transfer the irradiated sample of rock from the silica tube used for the irradiation to a nickel crucible containing sodium peroxide, mix the two powders intimately, and heat in an oven at 480° ± 10° C for 10 minutes. Remove the crucible from the oven, empty its contents carefully into a beaker containing 5 ml of carrier solution, wash out the crucible, and add the washings to the contents of the beaker. Add a few drops of hydrochloric acid, sp.gr. 1·18, to obtain a clear solution, warm, and then add ammonia solution, sp.gr. 0·880, to precipitate hydroxides. Separate the precipitated hydroxides from the supernatant liquid by centrifugation, discard the liquid, and dissolve the precipitate in the minimum amount of 72 per cent. perchloric acid. Transfer the solution to a beaker, and heat until fumes of perchloric acid are evolved to precipitate all silica.

Adjust the pH of the contents of the beaker to about 5 by adding 2 N sodium acetate, and filter through glass-wool into a 100-ml separating funnel. Shake the filtrate with a few millilitres of chloroform to saturate the aqueous phase, discard the excess of chloroform, and allow the aqueous phase to run through a column of dithizone. Wash the column with 50 ml of acetate buffer solution, 100 ml of EDTA solution and 100 ml of dilute perchloric acid, and elute the indium with the N hydrochloric acid. Collect 150 ml of eluate as soon as hydrochloric acid begins to leave the column.

Make the eluate alkaline with ammonia solution to precipitate indium hydroxide, boil to complete the precipitation, spin in a centrifuge, and reject the supernatant liquid. Dissolve the precipitated indium hydroxide in the minimum amount of hydrochloric acid, sp.gr. 1·18, dilute to about 25 ml, and add 3 ml of oxine solution. Warm to about 60° C, and add ammonium acetate solution to precipitate indium oxinate. Spin in a centrifuge, discard the supernatant liquid, and wash the precipitate with hot 5 per cent. ethanol. Make the precipitate into a slurry with a little 96 per cent. ethanol, transfer the slurry to a weighed aluminium counting tray, and remove the ethanol by evaporation under an infra-red lamp. Weigh to determine the chemical yield, and measure the activity of the sample with a scintillation counter.

Treatment of standards—

Transfer the standard to a 100-ml calibrated flask with a little 50 per cent. v/v hydrochloric acid, and dilute to the mark with de-ionised water. Add a suitable aliquot of this solution to 5 ml of carrier solution, then add 2 ml of 72 per cent. perchloric acid, and heat until fumes of perchloric acid are evolved to achieve exchange between active and inactive indium. Dissolve the residue in water, and continue as described under "Procedure," beginning at "Make the eluate alkaline with ammonia solution. . . ."

A separate series of experiments showed that the gamma-ray spectrum and specific activity of indium oxinate prepared by this method were similar to those obtained when the

indium standard and carrier were precipitated as hydroxide and then treated as described under "Procedure," beginning at "Separate the precipitated hydroxides from the supernatant liquid. . . ."

Discussion of the Method

After irradiation for 1 hour, the proportion of the total activity of a sample that was due to indium was extremely low. Consequently, it is probable that any failure of the subsequent procedure to isolate indium from all other active elements would lead to a significant contribution from impurities to the final measured activity. Differences would also be observed between the gamma-ray spectra and half-lives of the indium separated from the samples and from the standards.

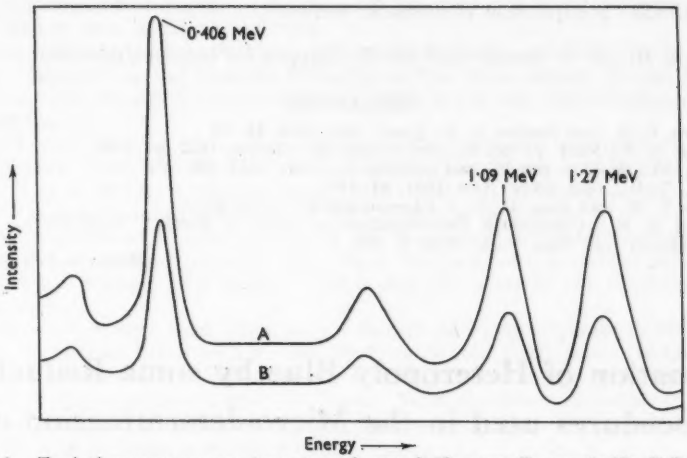

Fig. 1. Typical gamma-ray spectra : curve A, standard; curve B, sample No. E.G. 4327

There are certain differences between the extraction of a metal from an aqueous phase by dithizone retained on a column of cellulose acetate and by dithizone dissolved in an organic solvent, but the over-all pattern of extraction appears to be similar. For example, the dithizonates most stable to acid in liquid - liquid systems are formed by metals such as silver, mercury and palladium, and these require the most concentrated acid for elution from a column of dithizone. Elements likely to interfere in the final determination and which are extracted from the aqueous phase at pH 5 during the procedure and subsequently eluted by N hydrochloric acid are zinc and cadmium. The activity induced in these elements after irradiation for 1 hour is considerably less than that of the indium, and any interference from zinc and cadmium is further reduced by washing the column with EDTA solution, which decreases the ratio of both zinc and cadmium to indium on the column. No trace of zinc was observed in the final measured activity, even for the diabase W-1, in which the ratio of zinc to indium exceeded 1000 to 1.

The indium contents found when the proposed method was applied to various samples of rocks are shown below, each result being the mean of three or more determinations.

Sample	E.G. 4327	W-1	G-1	Canyon Diablo
Average indium content, p.p.m.	0·152 ± 0·001	0·055 ± 0·003	0·025 ± 0·002	0·012 ± 0·001

Decay curves of the indium derived from samples and standards indicated that the indium had been isolated in a radiochemically pure state, and this was confirmed by the gamma-ray spectrograms, for which the peak-height ratios calculated for the 0·406-, 1·09- and 1·27-MeV gamma rays were always similar for both standards and samples; examples of typical gamma-ray spectrograms are shown in Fig. 1.

The indium contents found by the proposed method compare favourably with those previously reported for E.G. 4327, W-1 and G-1 (0·169 ± 0·004, 0·064 ± 0·003 and 0·026 ±0·002 p.p.m., respectively), which were obtained by measuring the ^{116}In activity after separation by a more complicated procedure involving precipitation and solvent-extraction stages.

The chemical purity of the separated indium was not investigated, because of the relatively large amounts of carrier added, but the final precipitate of indium oxinate was always yellow, even for the meteorite, which contained more than 90 per cent. of iron. Overall chemical yields depended on the rate of flow of the liquid phase through the column during extraction and elution and on the amount of solvent retained by the solid phase, but yields were at least 50 per cent. and sometimes better than 85 per cent.

The time taken for a complete determination was dependent on the time needed for solution of the sample, but, if a rapid result was desired, the indium in the eluate from the column could be precipitated immediately as oxinate, the precipitation as hydroxide being omitted. This permitted counting to be started less than 30 minutes after the beginning of the separation (treatment with chloroform at pH 5). However, this led to lower yields, and the hydroxide precipitation is normally included.

We thank Mr. A. A. Smales and Mr. D. Mapper for helpful discussion.

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Formation of Heteropoly Blue by some Reduction Procedures used in the Micro-determination of Phosphorus*

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Reducing agents that react rapidly at room temperature have been compared with hydrazine sulphate, and generalities about the optimum concentrations of perchloric acid, ammonium molybdate and reducing agents have been tested as functions of the wavelength of measurement. Changes in the ratio and concentrations of sulphite and bisulphite have been shown to affect both intensity and stability of colour. The nature of the molybdophosphate complex is discussed.

The production of "heteropoly blue" is widely used for determining phosphorus colorimetrically, and, in general, the conditions used by various workers for forming the heteropoly acids of phosphorus and molybdenum have been remarkably uniform. In the subsequent reduction step, however, there have been great variations in temperature and in the nature and concentration of reducing agent used. Moreover, the optical density of the molybdenum blue has been measured at a variety of wavelengths, and the large number of procedures and chemicals available can confuse an analyst inexperienced in the determination.

An attempt has therefore been made to determine the nature of the heteropoly acid formed under standard conditions and to assess the virtues of some of the commonly used reducing agents and reduction procedures. On the assumptions that a uniform molecular species is reduced and that reducing agents similar in chemical nature produce the same heteropoly blue, 1,2,4-triaminonaphtholsulphonic acid, *p*-methylaminophenol sulphate (Elon

* *Contribution No.* 32.

or Metol) and 2,4-diaminophenol dihydrochloride (Amidol) have been compared at constant time, concentration and wavelength. (Throughout this paper, these compounds will be referred to as ANS, MAPS and DAPH, respectively.) Further, the precision and accuracy of the results obtained with colorimeters have been compared with those obtained with a spectrophotometer.

Materials and Methods

The phenolic reducing agents were obtained from the Eastman Kodak Co., New York; MAPS and DAPH were used without further purification, but the ANS was recrystallised before use.[1] The hydrazine sulphate and other inorganic chemicals used were of analytical-reagent grade; sodium metabisulphite was used whenever sodium bisulphite was required. For calculating concentrations of perchloric acid, densities of 1·6 and 1·5 were assumed for the 70 and 60 per cent. acids, respectively.

Stannous chloride and molybdate - hydrazine sulphate solutions were prepared as required.[2,3] Solutions of the phenolic reducing agents were allowed to age for 24 hours before use and were discarded when 1 week old; when not in use, the reducing solutions were kept in a refrigerator.

Standard conditions for comparing spectra of the unreduced and reduced forms of the molybdophosphate complex were selected as follows: perchloric acid, 0·92 M; ammonium molybdate, $2{\cdot}04 \times 10^{-2}$ M; orthophosphate, $5{\cdot}16 \times 10^{-5}$ M; reducing agent, $3{\cdot}3 \times 10^{-4}$ M in $4{\cdot}0 \times 10^{-3}$ M sodium sulphite and $3{\cdot}0 \times 10^{-2}$ M sodium bisulphite.

For spectrophotometric examination of the unreduced molybdophosphate complexes, the acidic aqueous solutions prepared by various methods were extracted with the solvent recommended by Wadelin and Mellon,[4] which was also used in the reference cell of the spectrophotometer.

Two colorimeters were used; these were a Bausch and Lomb Spectronic 20 and a Klett - Summerson colorimeter fitted with an S66 filter, calibrated Klett tubes (16 mm o.d.) being used in both instruments. A Beckman DU spectrophotometer and a Warren Spectracord equipped for repetitive scanning were also used. With the latter instrument, the solutions were scanned between 1100 and 550 mμ at 1-minute intervals. The wavelength setting of each was adjusted by using a mercury lamp.

Identification of the Molybdophosphate Complex

Because reaction conditions affect the type of molybdophosphate complex formed,[5] it was considered advisable to summarise, in standard units, the conditions used by the major proponents of the common reducing agents (see Table I); the standard conditions described above were selected on this basis.

Table I

Conditions used in various methods

Method No.*	1	2	3	4	5
Acid used	Perchloric	Perchloric	Perchloric	Sulphuric	Hydrochloric
Concentration of acid, M	0·92	0·46	0·72	0·5	0·65
Concentration of molybdate solution, M	$2{\cdot}04 \times 10^{-2}$	$1{\cdot}02 \times 10^{-2}$	$1{\cdot}69 \times 10^{-2}$	$1{\cdot}03 \times 10^{-2}$	$2{\cdot}43 \times 10^{-3}$
Concentration of phosphate, M	$5{\cdot}16 \times 10^{-5}$	$5{\cdot}16 \times 10^{-5}$	$5{\cdot}16 \times 10^{-5}$	$9{\cdot}69 \times 10^{-6}$	$5{\cdot}16 \times 10^{-5}$
Ratio of acid to molybdate	62·8	62·7	71·0	97·1	267
Ratio of molybdate to phosphate	395	197	327	1062	47·1
Reducing agent used	ANS	MAPS	DAPH	Hydrazine sulphate	Stannous chloride
Concentration of reducing agent, M	$3{\cdot}31 \times 10^{-4}$	$1{\cdot}16 \times 10^{-3}$	$4{\cdot}06 \times 10^{-3}$	$4{\cdot}60 \times 10^{-4}$	$2{\cdot}43 \times 10^{-3}$
Ratio of reductant to complex	0·53	1·87	6·55	39·7	3·92

* The methods used were—
1. Local variant of King's method.[6]
2. Harris and Popat's method.[7]
3. Allen's method.[8]
4. Beveridge and Johnson's method.[2]
5. Dickman and Bray's method.[3]

A comparison was made between the visible and ultra-violet spectra of the unreduced molybdophosphate complexes prepared as described by Wu[9] and by Wadelin and Mellon[4]

and under the standard conditions used in this investigation. The butanol - chloroform extracts of these complexes exhibited no appreciable absorption of light of wavelength longer than 400 mμ and all showed a broad peak at 310 to 315 mμ. When the aqueous solutions of the complexes were boiled for 10 minutes before extraction, the absorption at 310 mμ was decreased by about 90 per cent. and that at 265 mμ, near the "cut-off" point of the solvent, was increased by a factor of 2.

From the similarity of the spectra of the material prepared as described by Wu[9] and of the complex prepared in this investigation to the curves reported by Wadelin and Mellon[4] for phosphorus 12-molybdate, it was concluded that the standard procedure used here produced the dodecamolybdate complex. It was assumed, after a comparison of the experimental conditions summarised in Table I, that the other methods would also produce this complex.

Reduction of phosphorus 12-molybdenum complex by various reagents

Because little is known about the extent of reduction of the peripheral molybdenum atoms of the complex, reference absorption spectra of the heteropoly-blue colours produced by strong reducing agents were measured. Both stannous chloride at room temperature and hydrazine sulphate at 95° to 100° C gave spectra having sharp peaks at 815 mμ that were skewed toward the shorter wavelengths. The molecular extinction coefficients of the complexes produced by the action of stannous chloride and hydrazine sulphate were, respectively, $2{\cdot}64 \times 10^4$ and $3{\cdot}23 \times 10^4$.

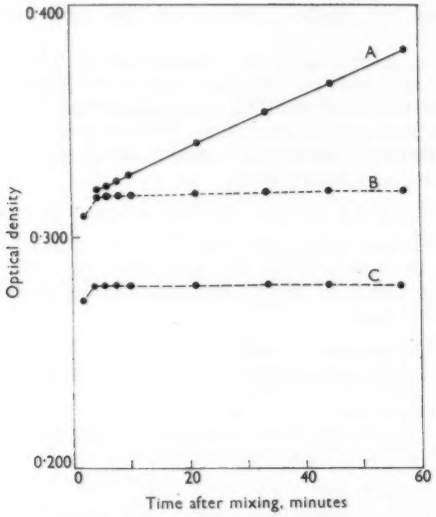

Fig. 1. Changes in optical density of ANS-developed blue as a function of time after mixing solutions. Measurements made at: curve A, 820 mμ; curve B, 720 mμ; curve C, 660 mμ

The intensity of colour developed by phenolic reducing agents is commonly measured at 660 or 820 mμ, and to ascertain whether or not the use of either wavelength gave equivalent information, the spectrum produced by ANS was compared with those formed by MAPS and DAPH over a period of 1 to 60 minutes after the beginning of colour development. In Fig. 1, the optical densities at three different wavelengths of an ANS-developed blue are shown as a function of time after the solutions were mixed. After the first 5 minutes, the absorptions at 660 and 720 mμ remained essentially constant for the remaining 55 minutes of the test; at 820 mμ, however, the value continuously increased. Equivalent information is shown in Fig. 2, in which transmittance at three intervals of time is plotted as a function

of wavelength. The blue colours developed by MAPS and DAPH were also stable at 660 and 720 mμ, but the rate of change of optical density at 820 mμ was only 10 per cent. of that of the ANS-developed solution.

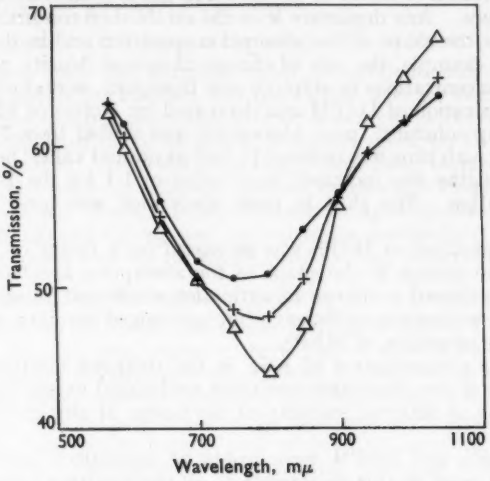

Fig. 2. Repetitive scanning of spectrum of ANS-developed blue: •, 2 minutes after mixing solutions; +, 21 minutes after mixing solutions; △, 56 minutes after mixing solutions

TABLE II

EXTINCTION COEFFICIENTS AFTER 10 MINUTES

Reducing agent	Molecular extinction coefficient × 10^{-3} at—		
	660 mμ	720 mμ	820 mμ
ANS	5·39	6·16	6·34
MAPS	4·94	5·56	5·37
DAPH	5·16	5·72	5·52

TABLE III

EFFECT OF TIME ON OPTICAL DENSITY DEVELOPED BY PHENOLIC REDUCING AGENTS UNDER STANDARD CONDITIONS

The conditions used were: concentration of reducing agent, $3{\cdot}3 \times 10^{-4}$ M; concentration of perchloric acid, 0·92 M; concentration of sodium sulphite, $4{\cdot}0 \times 10^{-3}$ M; concentration of sodium bisulphite, $3{\cdot}0 \times 10^{-2}$ M; concentration of molybdate, $2{\cdot}04 \times 10^{-2}$ M; concentration of orthophosphate, $5{\cdot}16 \times 10^{-5}$ M

Reducing agent	Measurements at 660 mμ		Measurements at 720 mμ		Measurements at 820 mμ	
	Rate of increase of absorption, units per minute	Optical density after 20 minutes	Rate of increase of absorption, units per minute	Optical density after 20 minutes	Rate of increase of absorption, units per minute	Optical density after 20 minutes
ANS	$7{\cdot}00 \times 10^{-4}$	0·312	$1{\cdot}27 \times 10^{-3}$	0·352	$3{\cdot}25 \times 10^{-3}$	0·373
MAPS	$6{\cdot}00 \times 10^{-4}$	0·237	$8{\cdot}00 \times 10^{-4}$	0·267	$1{\cdot}50 \times 10^{-3}$	0·287
DAPH	$1{\cdot}10 \times 10^{-3}$	0·248	$1{\cdot}30 \times 10^{-3}$	0·278	$1{\cdot}45 \times 10^{-3}$	0·279

After development of colour for 10 minutes, the extinction coefficients of the three blues were as shown in Table II. That for the ANS-developed blue at 660 mμ, which was

stable for 60 minutes, is equal to that of the MAPS-developed blue at 820 mμ and compares well with the value for DAPH, provided that freshly recrystallised ANS is used.

Experiments were next made to determine the effect on the absorption spectra of changing the concentration of buffer or the reducing agent from the values commonly recommended in the literature. Any departure from the established conditions for a given reducing agent was reflected in the shape of the absorption spectrum and in the rate at which colour was developed. For example, the rate of change of optical density at 820 mμ was doubled for ANS when the concentrations of sulphite and bisulphite were doubled.

When the concentration of DAPH was decreased by factors of 12 and 120 (the concentration of buffer being constant), peak absorption was shifted from 720 to 820 mμ and the stability of the colour with time was reduced to half its normal value; however, optical density at any of the wavelengths was increased by a factor of 1·1 for the 1-to-12 dilution and 1·2 for the 1-to-120 dilution. The shift in peak absorption was most pronounced with the most dilute solution.

When the concentration of MAPS was increased by a factor of 1·4 or decreased by a factor of 0·33 there no change in the shape of the absorption spectrum. However, the increased concentration caused a change in extinction coefficient by decreasing the stability of the colour. Lower extinction coefficients, but unchanged stability, were the consequences of decreasing the concentration of MAPS.

A decrease in the concentration of ANS in the reducing mixture by a factor of 0·33 left the over-all shape of the absorption spectrum unchanged except for a decrease of 10 per cent. in the absorption at 820 mμ; stability of the colour at any given wavelength was not greatly affected.

When ANS, MAPS and DAPH were tested at equivalent concentrations under the "standard" conditions used in this investigation, all the resulting heteropoly blues were less stable than those produced under prescribed conditions; the results are summarised in Table III. The ANS-produced absorption at 660 mμ was as stable as the absorptions at 820 mμ developed by the other two phenolic reducing agents and was more intense.

Effect of heat on development of heteropoly blue

Beveridge and Johnson's[2] hydrazine sulphate system was chosen to give a reference spectrum for heated solutions. Reaction mixtures were heated at 60° C for 6, 12, 18 or 60 minutes, and their spectra were compared with that of a solution containing hydrazine sulphate and that had been heated in a boiling-water bath for 5 minutes. The spectrum of a mixture heated for 6 minutes was broad and flat; after being heated for 12 minutes at 60° C, however, the mixture had a spectrum resembling that of the boiled solution (a prominent shoulder-less peak at 820 mμ), and the spectrum of a mixture heated for 60 minutes exhibited 85 per cent. of the absorption (at 820 mμ) of the boiled solution. At no time did the spectra resemble those obtained with MAPS or DAPH at room temperature. The absorption at 660 mμ of the boiled solution was 40 per cent. of its value at 820 mμ.

Table IV

Extinction coefficients under various conditions

Conditions	Molecular extinction coefficient × 10^{-4} for—					
	ANS		MAPS		DAPH	
	At 660 mμ	At 820 mμ	At 660 mμ	At 820 mμ	At 660 mμ	At 820 mμ
Prescribed	1·40	3·52	1·56	3·84	1·42	3·28
Standard	0·85	2·81	0·59	1·85	1·11	3·45

Heating the reaction mixture has been considered a source of accidental error.[7] This was to some extent confirmed when paired samples were heated for increasing lengths of time to determine the time of heating for optimum development of colour. With both ANS and MAPS, heating for longer than 7 minutes produced increasing differences between the pair of samples. With ANS, heating for 6 minutes was sufficient, but solutions reduced by MAPS did not achieve stability of absorption before disagreement between duplicates occurred.

Reducing solutions containing ANS, MAPS and DAPH in "standard" and prescribed amounts were heated for 10 minutes in a boiling-water bath.[1,8,10] All six spectra had well defined peaks at 820 mμ, with the extinction coefficients shown in Table IV.

On average, the ratio between the absorptions at 660 and 820 mμ was 0·42 for the regular solutions and 0·32 for the standard solutions.

Effect of sequence of addition of reagents on spectral properties

Allen[8] recommended that the reducing agent (DAPH) be added to the test mixture before the ammonium molybdate solution, but most other workers suggest that the reducing agent be added last. Because determination may be interrupted after the molybdate solution has been added, the effects of delay in adding reducing agent on the resulting absorption spectra were studied briefly. With Allen's system, containing molybdate, delays of up to 17 minutes in adding the reducing solution affected neither the shape of the spectrum nor the intensity of absorption. When the addition was delayed for 34 minutes, however, the optical densities at 660, 720 and 820 mμ were increased, on average by 5 per cent. A 78-minute delay in adding the solution of ANS or MAPS produced no detectable change.

Choice of instrument and reducing solution

Six solutions containing potassium phosphate equivalent to from 1 to 40 μg of phosphorus were analysed by the ANS method described here and by the MAPS method of Harris and Popat.[7] The intensities of the heteropoly-blue colours were measured with a Klett - Summerson colorimeter at essentially 660 mμ and with a Beckman DU spectrophotometer at 660, 720 and 820 mμ. The results were statistically analysed to determine which of the two reducing systems gave the better results and whether or not the colorimeter compared favourably with the spectrophotometer. The residual variances and standard errors were such that the use of a Klett colorimeter for measuring the absorption of ANS-produced blue at 660 mμ should give results as accurate as those obtained with MAPS and a spectrophotometer at 820 mμ. A similar assessment of the quality of results for colours developed with heating showed that a Spectronic 20 colorimeter operated at 820 mμ gave results as useful as those obtained with the DU spectrophotometer at the same wavelength.

Discussion of results

The nature of the molybdophosphate complex reduced in these determinations has been debated. Wu[9] prepared the 12- and 9-molybdate complexes of phosphorus and showed that the conditions for their preparation were markedly different. Berenblum and Chain,[10] however, dismissed the significance of Wu's work and stated that the various heteropoly acids of phosphorus and molybdenum exist in solution in equilibrium. In none of the recent studies of heteropoly-acid inter-conversion,[11,12] however, was inter-conversion between the 12- and 9-molybdate forms considered in the absence of large amounts of free orthophosphate.

Reducible heteropoly acids of phosphorus and molybdenum have been considered to exist in either colourless or yellow forms,[10,13] and Ferrari[13] has developed the hypothesis that different reducing agents act on the different forms of the complex to produce heteropoly-blue colours having characteristic spectra. In our work, however, subtle changes in reaction conditions were found to be sufficient to produce either a colourless or a yellow solution of the molybdophosphate complex. Wu's preparations were always highly coloured, even when diluted 1000 times (by which the concentration of the hydrochloric acid in the preparation was decreased to 0·003 N). Complexes prepared acording to Dickman and Bray[3] and Wadelin and Mellon,[4] on the other hand, were always colourless, even although the acidities and concentrations were not greatly different from those of the coloured solutions. Ferrari's preparations[13] were sometimes coloured and sometimes colourless.

Some doubt was cast on Ferrari's hypothesis by (*a*) the observation that colourless solutions when extracted with Wadelin and Mellon's solvent gave yellow extracts and (*b*) the common absorption peak at 310 to 320 mμ exhibited by all the extracts. Absorption of this type has been assigned by Wadelin and Mellon[4] to the 12-molybdate complex.

An alternative, or possibly additional, consideration is that more than one species may contribute to the blue colour formed by other than the strongest reducing agents under optimum conditions. Table I shows that a molar ratio of reducing agent to molybdenum of less than 12 to 1 is used in some systems, and in a recent monograph on the chemistry of phosphorus[5] it is stated that the extent of reduction of peripheral molybdenum in complexes

is not known with certainty. Further, in Fig. 2 there is a suggestion of the existence of two isosbestic points, at 660 and 900 mμ. This evidence would seem to indicate that heteropoly blue is indeed heterogeneous, and further investigation of its chemical nature would be of great value in standardising the reduction procedure used in the determination of phosphorus.

When selecting operating conditions for determining phosphorus, a balance must be struck between stability of the colour with time and the magnitude of the extinction coefficient of the heteropoly blue. This investigation has shown that stability of colour intensity is so easily lost that established methods should be adhered to scrupulously. The use of MAPS or DAPH in place of ANS should also be viewed cautiously because of the lower extinction coefficient of the resulting heteropoly blue. The stability and extinction coefficient of the ANS-produced colour at 660 mμ were equal to those of the MAPS- or DAPH-developed blues at 820 mμ. Moreover, 660 mμ is within the range of all colorimeters, whereas detection of absorption at 820 mμ requires the use of a red-sensitive photocell. If red-sensitive photocells are available, however, the wavelength of the isosbestic point, 900 mμ, would be the logical wavelength for measuring the colour; at 900 mμ, all reduced species absorb equally.

The careful technical assistance of Mr. J. C. Mes during part of this investigation is gratefully acknowledged.

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The Determination of Fluorine in Deposit-gauge Samples

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The reaction between fluorine, cerium and alizarin complexone described by Belcher, Leonard and West is used for determining fluorine in samples from deposit gauges. Prior separation from interfering elements is achieved by means of a column of cation-exchange resin and also by distillation from perchloric acid solution. The results obtained by these two methods agree well with those found by a method involving distillation from perchloric acid and subsequent titration with thorium nitrate solution (alizarin red S as indicator).

The possibility of developing a simple and rapid procedure for determining fluorine in samples from deposit gauges was suggested by the work of Belcher, Leonard and West,[1,2] who used the reaction with cerium and alizarin complexone to determine fluorine in organic compounds. Previous unpublished work at the Fuel Research Station had established that the fluorine contents of these samples collected over a period of 1 month in industrial areas (Greenwich, Sheffield and Stoke-on-Trent) amounted to a total of 1 to 2 mg, the dilution depending on the rainfall during the period. Further, it was established that all but a small proportion

of the total fluorine in the sample appeared in the liquid phase. In this investigation, the samples were taken from a polythene gauge and were filtered through a Whatman No. 1 filter-paper before use. The residues, sometimes containing appreciable amounts of organic matter, were discarded.

Experimental

An investigation of the conditions under which the complex of fluorine with cerium and alizarin complexone is formed showed that the intensity of the colour attains a maximum value after approximately 30 minutes, and no difference could be detected between solutions that had been set aside for this period on the bench away from direct sunlight and those that had been stored away from light and draughts.

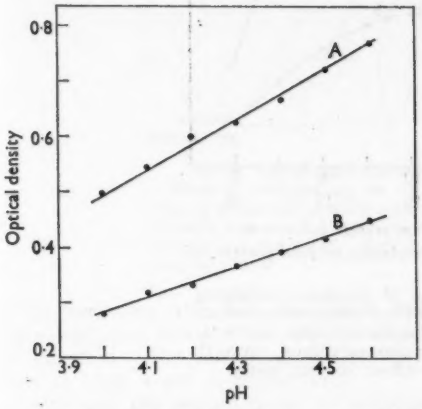

Fig. 1. Graphs showing increase in optical density with pH: curve A, solution containing 25 μg of fluorine, 2 ml of buffer solution (pH 4·3) and 10 ml each of alizarin complexone and cerous nitrate solutions in 100 ml; curve B, reagent blank solution

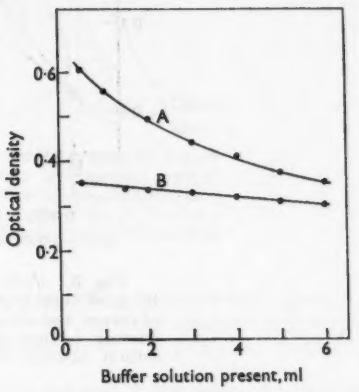

Fig. 2. Graphs showing variation in optical density with concentration of sodium acetate-acetic acid buffer solution (pH 4·3): curve A, solution containing 20 μg of fluorine per 100 ml; curve B, reagent blank solution

In the initial experiments, the pH of each solution was carefully adjusted to 4·3, and, as the importance of making all optical-density measurements at a pre-determined pH was clearly demonstrated, this value was adhered to in all subsequent work. Fig. 1 shows the relationship between optical density and pH for solutions containing 25 μg of fluorine per 100 ml. At high pH values, the optical densities of both coloured and reagent blank solutions increased considerably, and for this reason the pH of 4·3 suggested by Belcher, Leonard and West was preferred. All measurements of optical density were made with a Spekker absorptiometer fitted with a tungsten lamp and No. 6 filters. The optical densities of these solutions are also dependent on the concentration of buffer or salt present; this is shown by Fig. 2, in which the optical densities of solutions containing 20 μg of fluorine per 100 ml are plotted against the volume of buffer solution present.

Attempts were made to determine the composition of the complex by the method of "continuous variation." Several solutions were prepared, each containing the same amount of fluorine, but different amounts of cerous and alizarin complexone solutions. The optical densities of these solutions are shown in Fig. 3, together with those of the reagent blank solutions containing no fluorine. It can be seen from Fig. 3 that, in the absence of fluorine, cerium and alizarin complexone are combined in the ratio of 1 to 1.

In the presence of fluorine, maximum optical densities are obtained when the ratio of concentrations of cerium to alizarin complexone is of the order of 1·2 to 1. In order to establish this value more closely and to include fluorine as a separate variable, the continuous-variation diagram was extended to three dimensions by plotting the optical densities as contours on a triangular diagram. This is shown in Fig. 4, which is based on measurements

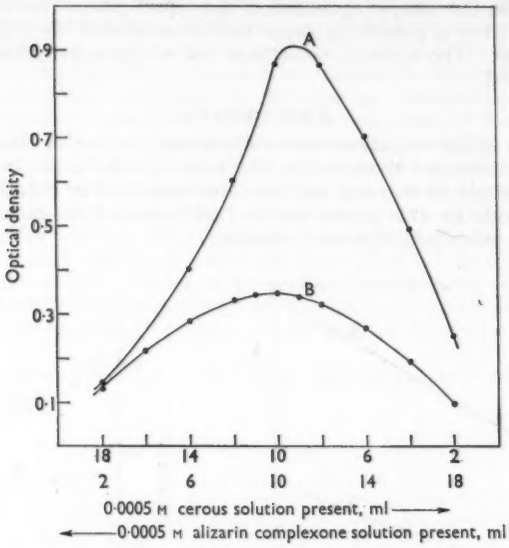

Fig. 3. Optical densities of solutions containing 100 μg of fluorine per 100 ml, with variations in amounts of cerous and alizarin complexone solutions: curve A, fluorine - cerium - alizarin complexone colour; curve B, cerium - alizarin complexone colour (reagent blank)

from 144 solutions containing different amounts of cerium, fluorine and alizarin complexone solutions. The maximum optical-density values were obtained in the region corresponding to a composition whose simplest ratio was—

$$(\text{fluorine})_4 : (\text{cerium})_5 : (\text{alizarin complexone})_4$$

This composition is not unreasonable. It could be explained by postulating a large molecular species, in which four ligand groups surround a central cerium atom, the ligand residues being linked through additional cerium atoms; for such a structure the size of the central atom would be critical. In this connection, it may be noted that Leonard and West[3] have found that lanthanum and praseodymium react to form similar blue complexes. Greenhalgh and Riley[4] have reported that, of the complexes of the lanthanide elements with fluorine and alizarin complexone, the maximum optical-density values are obtained with lanthanum itself. Some increase in sensitivity to fluorine can be obtained by using cerium and alizarin complexone solutions in the ratio indicated, *i.e.*, 1·25 to 1, and also by replacing the cerous solution with one of lanthanum.

TABLE I

COMPARISON OF RESULTS BY PHOTOMETRIC AND STANDARD METHODS

Sample No.	Concentration of fluorine found by— direct photometric measurement, p.p.m.	distillation and titration, p.p.m.
SW4	0·30, 0·31	0·14, 0·14, 0·23 0·15, 0·16
SW8	0·44, 0·47	0·32, 0·35
SW15	0·42	0·25, 0·25

Ions known to interfere with the determination include all those metal ions that compete with cerium for the alizarin complexone, all those that themselves form complexes with

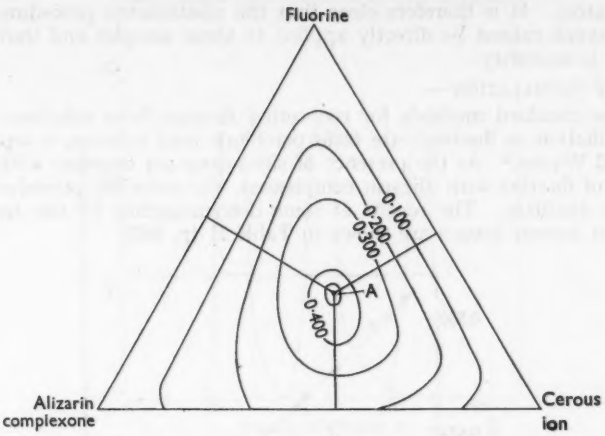

Fig. 4. Continuous-variation diagram for the system $F^- + Ce^{3+}$ + alizarin complexone = 15 ml of 0·0005 M solution; optical density measured in 4-cm cells with a Spekker absorptiometer (No. 6 filters) and plotted as contours using intervals of log (I_0/I) of 0·1, 0·2, 0·3, 0·4 and 0·425. Position marked A indicates a composition ratio of 4:4:5

fluoride (including Al^{3+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) and the anions arsenate, phosphate, citrate, tartrate, oxalate and ethylenediaminetetra-acetate[2]; neither chloride nor sulphate ions interfere. The interferences from iron and aluminium in the proposed method are shown in Figs. 5 and 6, respectively.

Although the concentration of interfering elements present in samples of water from deposit gauges was known to be small, it was not possible to apply the proposed procedure directly to these samples. This is shown by Table I, in which the results of a number of such determinations are compared with those found by a standard method (distillation from perchloric acid solution and subsequent titration with thorium nitrate solution, with alizarin

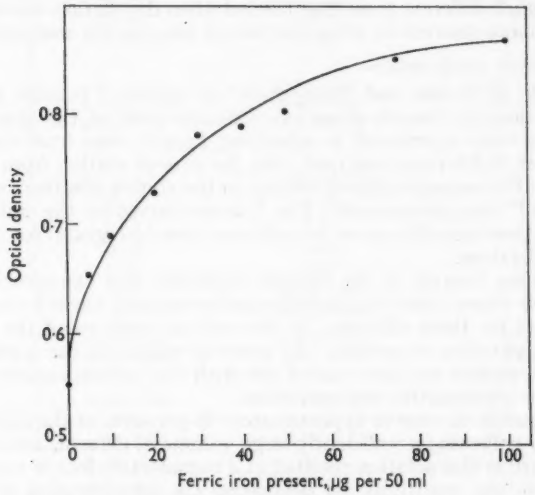

Fig. 5. Effect of iron on optical density of a solution containing 10 µg of fluorine per 50 ml

red S as indicator). It is therefore clear that the photometric procedure involving use of alizarin complexone cannot be directly applied to these samples and that prior separation of the fluorine is necessary.

SEPARATION BY DISTILLATION—

One of the standard methods for recovering fluorine from solutions containing other ions is by distillation as fluorosilicate from perchloric acid solution, a separation described by Willard and Winter.[5] As the presence of silica does not interfere with the photometric determination of fluorine with alizarin complexone, the proposed procedure can be applied directly to the distillate. The results of some determinations by this method of fluorine in samples from deposit gauges are shown in Table II (p. 595).

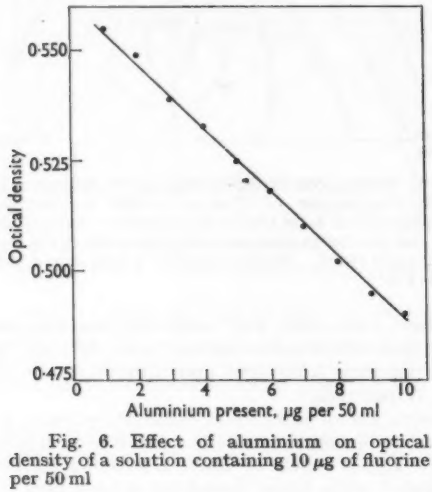

Fig. 6. Effect of aluminium on optical density of a solution containing 10 μg of fluorine per 50 ml

In view of the time required for distillations, however, attempts were made to use an ion-exchange resin for this separation. The time needed for a single separation by ion exchange was not much different from that needed when distillation was used, but separation by ion exchange is more convenient when batches of samples are analysed.

SEPARATION BY ANION EXCHANGE—

From the work of Nielsen and Dangerfield,[6] it appeared feasible to separate fluorine from interfering cations by means of an ion-exchange resin of the quaternary ammonium type. No difficulty was experienced in adsorbing fluoride ions from standard solutions of fluoride when Dowex 1–X8 resin was used, but the rate of elution from the resin depended to a great extent on the concentration of acetate in the eluting solution; with dilute solutions the tendency to "tail" was pronounced. Fig. 7 shows curves for the elution of fluorine from Dowex 1–X8 resin; these smooth curves were drawn from histograms for three concentrations of sodium acetate solution.

When determining fluoride in the effluent, difficulty was experienced in adjusting the pH, which, as shown above, must be carefully and accurately carried out. New calibration curves were required for these effluents, as the optical densities of the fluoride complexes depend on the concentration of acetate. In order to reduce to the minimum the difficulty in adjusting the pH, elution was also carried out with the sodium acetate - acetic acid buffer solution used in the photometric determination.

It was found possible to recover approximately 95 per cent. of the added fluorine (usually 100 μg), but only by collecting a sufficiently large volume of effluent, and the high concentration of sodium acetate in this solution resulted in a considerable loss in sensitivity to fluorine. Attempts to increase the sensitivity by decreasing the concentration of buffer resulted in increased dispersion of the added fluorine and lower recovery of fluorine from any selected portion of the effluent. This difficulty could be resolved by collecting a volume of effluent

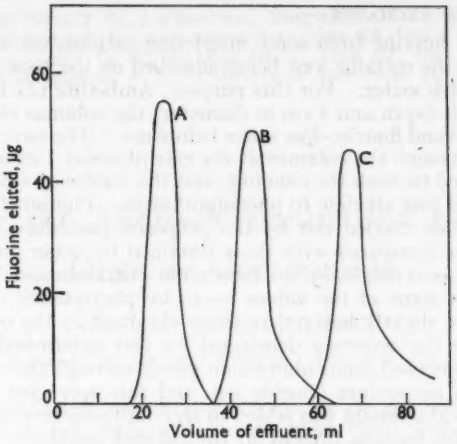

Fig. 7. Curves plotted from histograms for the elution of 100 µg of fluorine from a column of Dowex 1–X8 resin (10 cm × 1 cm diameter) by sodium acetate solution of molarity: curve A, 0·3; curve B, 0·2; curve C, 0·1

sufficiently large to contain at least 95 per cent. of the fluorine and subsequently using an aliquot of this solution containing the minimum amount of sodium acetate - acetic acid buffer. Unfortunately, this was not possible with the rain-water samples examined, many of which contained about 0·1 p.p.m. of fluorine.

TABLE II

COMPARISON OF RESULTS BY VARIOUS METHODS

Sample No.	Concentration of fluorine found by— distillation and titration, p.p.m.	distillation and photometry, p.p.m.	ion exchange and photometry, p.p.m.
SW8	0·32	0·34	0·35
	0·35	—	0·34
SW10	0·13, 0·13	0·15, 0·17	0·15, 0·15, 0·14
	0·15, 0·14	0·14, 0·14	0·15, 0·16, 0·16
	0·15, 0·15	0·14, 0·14	0·13, 0·14, 0·14
	—	—	0·15, 0·14, 0·11
	—	—	0·14, 0·12, 0·14
	(mean 0·14)	(mean 0·14)	(mean 0·14)
SW12	0·17	0·16	0·16
	0·17	0·16	—
SW14	0·09	0·09	0·08
	0·09	0·08	0·08
SW15	0·25	0·26, 0·24	0·20
	0·25	0·25, 0·24	0·20
SW16	0·23, 0·24	0·20, 0·20	0·18, 0·18
	0·20, 0·22	0·20, 0·22	0·21, 0·20
	—	—	0·20, 0·21
SW17	0·16, 0·13	0·15, 0·14	0·15
	0·17, 0·17	0·14, 0·14	0·14
	0·13, 0·14	0·13, 0·13	—
SW18	0·12, 0·12	0·11	0·15, 0·11
	0·11, 0·11	0·11	0·11, 0·11
SW19	0·54	0·51	0·44, 0·42
	0·53	0·52	0·42, 0·40

SEPARATION BY CATION EXCHANGE—

The separation of fluorine from some interfering cations was also achieved by using a cation-exchange resin, the metallic ions being adsorbed on the resin and the fluorine washed through the column with water. For this purpose, Amberlite CG-120 was used in columns approximately 10 cm in depth and 1 cm in diameter; the columns of resin were washed with dilute hydrochloric acid and fluorine-free water before use. The samples, filtered as described, were allowed to pass through the columns at the rate of about 1 drop per second. Fluorine-free water was then used to wash the columns, and the combined effluents were concentrated by evaporation at a pH just alkaline to phenolphthalein. Photometric determination of the fluorine present was then carried out by the proposed procedure. The results of several such determinations are compared with those obtained by other methods in Table II.

The agreement between results by the distillation - titration and distillation - photometric procedures is good, but some of the values found by photometric determination after ion-exchange separation are slightly lower than those obtained by the other two methods; they are nevertheless within the accuracy demanded for this determination.

Under the conditions used, some aluminium passes through the cation-exchange column, probably as a complex aluminium fluoride ion, and this may give rise to the low results. It was found that when aluminium was added to standard solutions of fluoride before passage through the column, the fluoride present in the effluent could not be directly determined photometrically, but could be recovered by distillation and then determined with alizarin complexone, indicating the presence of aluminium in the effluent. The possible presence of aluminium, with subsequent interference, must therefore be considered when the ion-exchange separation is used before photometric determination of fluorine by the proposed procedure.

METHOD

The procedure described below was finally adopted for the determination of fluorine in samples of water from deposit gauges.

REAGENTS—

These were prepared as described previously.[1] Fluorine-free water was prepared by passing distilled water through a column of anion-exchange resin.

PROCEDURE—

A 50- or 100-ml portion of the filtered sample from the deposit gauge is allowed to pass through a column of cation-exchange resin prepared as described above, at the rate of approximately 1 drop per second, and the column is washed with about 100 ml of fluorine-free water, elution being at the same rate. The effluents are combined, and 2 drops of phenolphthalein indicator solution and then extremely dilute sodium hydroxide solution are added until the coloured form of the indicator appears. The solution is then evaporated in a platinum vessel to approximately 50 ml and transferred to a 100-ml calibrated flask. Next, 2 ml of acetate buffer solution (pH 4·3), 10 ml of alizarin complexone solution and 10 ml of cerous nitrate solution are added in that order, and the solution is diluted to the mark and mixed well. The solution is then set aside for at least 30 minutes before its optical density is measured with a Spekker absorptiometer fitted with a tungsten lamp and No. 6 filters. The fluorine content of the sample is obtained by reference to a calibration graph plotted from the optical densities of coloured solutions containing 5 to 40 μg of fluorine per 100 ml prepared from a standard solution of sodium fluoride.

The novel nature of the alizarin complexone reaction and the need for a simple method for determining fluorine have resulted in a great deal of interest in this development of fluorine chemistry. We are particularly grateful to the following workers, who have supplied details of progress in this field: Dr. J. P. Riley, University of Liverpool, Mr. C. A. Johnson, Boots Pure Drug Co. Ltd., and Mr. J. R. W. Kerr, Laboratory of the Government Chemist.

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Methods for the Micro-determination of Sulphur in Organic Compounds

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Rapid methods for determining sulphur in the solutions obtained by combustion of organic compounds in an oxygen-filled flask are described. Interference from nitrate, chloride, bromide, phosphate and some metal ions has been overcome. For most samples, the sulphate is assayed by conductimetric titration with barium ions, for which a Pye conductance bridge (catalogue No. 11700) is used.

Recent literature includes numerous papers dealing with the application of combustion in an oxygen-filled flask to the micro-determination of elements. The products of combustion are absorbed in a suitable solution, in which the element of interest can be determined. If the test material is a simple one, containing, for example, only sulphur, carbon, hydrogen and oxygen, the sulphur can readily be determined as sulphuric acid by titration with sodium hydroxide solution. The combustion of compounds containing reactive elements other than sulphur produces a solution of mixed acidic ions, and the titration procedure must be selective towards sulphate ions. Specific methods for the determination of sulphate ions include titration with barium perchlorate solution,[1] gravimetric determination as barium sulphate, treatment of the precipitated barium sulphate with a strongly acid cation-exchange resin and subsequent titration of the liberated sulphuric acid[2] and precipitation with 4-amino-4′-chlorodiphenyl hydrochloride.[3] In all these methods, interference from phosphate ions may be encountered, and procedures for removing phosphate by precipitation and filtration are described. Methods involving separations, however, are never ideal for micro-determinations, and a method of direct treatment of the solution is to be preferred. Recently,

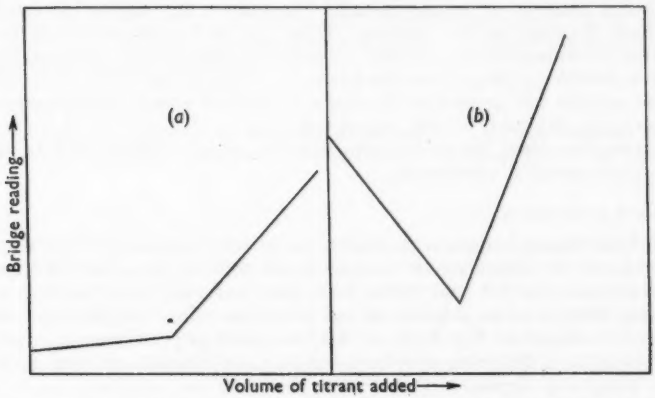

Fig. 1. Specimen graphs for titration of sulphate ion with solutions of (*a*) barium chloride and (*b*) barium acetate

methods based on the reaction of barium chloranilate with sulphate ions have been described,[4,5] and this colorimetric reaction has been used in conjunction with combustion in an oxygen-filled flask.[5]

Conductimetric titration with barium ion is well suited to the micro scale and especially to the solutions obtained from combustion in an oxygen-filled flask, since the volume is small and the concentration of ions is low. The sulphate ions are titrated with barium acetate solution, and the conductivity of the solution decreases, owing to precipitation of barium sulphate and replacement of the highly mobile sulphate ions by acetate ions of low mobility. After the equivalence point has been reached, excess of barium ion causes an increase in conductivity. Barium acetate is preferable to barium chloride as titrant, since the mobility of the acetate ion is much less than that of the chloride ion; a better end-point can therefore be obtained (see Fig. 1). A comparison conductivity cell is used in the reference arm of the bridge circuit, and the cell is immersed in the test solution to minimise changes in temperature. The use of a comparatively concentrated solution of the titrant delivered in small increments from a micro syringe-burette eliminates dilution effects. The conductivity of the solution is still further decreased and precipitation hastened by titrating in partly alcoholic medium. Neutralisation of the solution before titration is important. Conductimetric titration of a mixture of sulphuric and hydrochloric acids with barium acetate solution involves two effects: removal of sulphate ion by precipitation, and a concomitant decrease in the concentration of hydrogen ion as acetic acid is generated. The latter effect continues until the hydrochloric acid also is replaced by an equivalent amount of weakly dissociated acetic acid. The end-point is therefore a measure of the sum of the two acids. If the solution has first been neutralised with ammonia, the change in conductivity will be due only to the removal of sulphate ion as barium sulphate. Subsequent titration of chloride may be carried out with a standard solution of silver nitrate.

Phosphate ions, if present, will be precipitated as barium phosphate and will interfere with the determination of sulphate. This interference can be eliminated by adding silver nitrate solution to precipitate phosphate before titration with barium ions.

When a solution containing orthophosphoric acid is neutralised to methyl red with ammonia, the primary orthophosphate, $(NH_4)H_2PO_4$, is produced, and the ionic equilibrium existing in the solution will be—

$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-} \rightleftharpoons H^+ + PO_4^{3-}$$

Precipitation of phosphate as Ag_3PO_4 involves removal of PO_4^{3-} ions, with consequent displacement of the equilibrium and generation of acid. After sufficient silver nitrate to precipitate all the phosphate has been added, it is necessary to restore neutrality by adding more ammonia. The addition of a large excess of silver nitrate should be avoided, as this will mask the change in conductivity. In the presence of chloride or bromide (iodine is removed by boiling at an earlier stage), sufficient silver nitrate to precipitate both halide and phosphate ions must be added, otherwise removal of phosphate may be incomplete.

The successful analysis of organo-metallic compounds for sulphur by combustion in an oxygen-filled flask depends on two factors. First, the decomposition must be "clean" and not accompanied by formation of a firmly adhering deposit on the platinum wire; secondly, on combustion a soluble sulphate must be formed. Finally, the choice of a suitable method for determining sulphur will depend on the natures of other anions that may be present after the combustion, *e.g.*, chloride, nitrate, phosphate, etc.

The examples described below indicate ways in which sulphur can be determined in a variety of organo-metallic compounds.

POTASSIUM BENZENESULPHONATE—

Potassium benzenesulphonate was chosen as a test compound because the products of combustion would be expected to contain water-soluble potassium sulphate. Neutral hydrogen peroxide (methyl red - methylene blue indicator) was the absorbing medium, and, after combustion, there was no deposit on the platinum wire. A platinum wire bent into the shape of an S as shown in Fig. 3 (*b*), p. 601, was used in preference to a platinum gauze for holding the sample. This was simpler to inspect for deposit and easier to wash in the event of there being any deposit.

After combustion, the solution was allowed to percolate through a column of the strongly acid cation-exchange resin Amberlite IR-120(H) to replace potassium ions by hydrogen ions.

The solution was then boiled to expel carbon dioxide, and the sulphuric acid was titrated with 0·01 N sodium hydroxide. The results are shown in Table I.

COPPER SALT OF *p*-HEXADECYLOXYBENZENESULPHONIC ACID—

The method outlined above was used, and results are also shown in Table I.

CHLORAMINE-T—

Combustion and percolation to remove sodium ions were carried out as before, but the direct acidimetric titration was not applicable, owing to the presence of nitrogen and chlorine. The solution was boiled to expel carbon dioxide, neutralised to methyl red and titrated conductimetrically with barium acetate solution. In neutral solution, chloride and nitrate ions do not interfere with the titration of sulphate ion. These results are included in Table I.

TABLE I

SULPHUR CONTENTS FOUND IN ORGANO-METALLIC COMPOUNDS

Sample	Elements present	Sulphur content found, %	Theoretical sulphur content, %
Potassium benzenesulphonate	C, H, O, S and K	16·2	16·3
Copper salt of *p*-hexadecyloxybenzenesulphonic acid	C, H, O, S and Cu	7·4	7·5
Chloramine-T	C, H, O, S, N, Cl and Na	11·6	11·4
Zinc di(octylthioate)	C, H, O, S, P and Zn	16·3	16·3*

* Result found by using a Parr bomb.

ZINC DI(OCTYLDITHIOATE)—

Combustion and percolation to remove zinc ions were carried out as before. Both the acidimetric and the previously described conductimetric titration procedures were unsuitable because of interference from phosphate ions present in the solution. This difficulty was overcome by using the procedure described later, in which the interfering phosphate ions are first removed by precipitation as insoluble silver phosphate. Finally, the sulphate content was determined by conductimetric titration with barium acetate solution; the results are shown in Table I.

The organo-metallic compounds so far tested have contained sodium, potassium, copper and zinc. The sulphates of these metals are water-soluble, and it seems probable that all compounds giving rise to water-soluble metal sulphates can be handled in a similar manner. Solution of the products of combustion by other methods has not yet been attempted.

DESCRIPTION OF APPARATUS

CONDUCTANCE BRIDGE—

We have used a Pye conductance bridge, which, as well as providing a direct measurement of conductivity, permits comparison to be made between two conductivity cells. When necessary, the resistive balance is adjusted by connecting a variable capacitance across the electrodes dipping into the test solution.

ELECTRODE ASSEMBLY—

Two pairs of bright platinum-wire electrodes are used. The pair making contact with the test solution is bound to the outside of a test-tube containing the other pair, as shown in Fig. 2.

SYRINGE-BURETTE—

The capacity of the syringe-burette used in micro titrations is usually 0·5 ml, and increments as small as 0·001 ml can be delivered. In conductimetric titrations, however, delivery of such small increments is not normally required, since the readings in the vicinity of the end-point are often unreliable. The end-point is extrapolated from the readings before and after those in the region of it. Moreover, 0·5 ml of solution is frequently insufficient for a complete titration, and the possibility of calibrating syringes of larger capacity to deliver titrant with a similar precision has therefore been investigated.

Commercially available 2- and 3-ml syringes have been calibrated and found to be eminently suitable for this work. After slight modification of the barrel and plunger (by

removing the projecting flanges), they can be used in the normal vernier-gauge support. Calibration with water (by weight) showed that the precision of the bore was good and that a calibration factor could be determined from the weight of water expelled per revolution of the vernier; the factors for several different 2-ml syringes were found to be identical. When a syringe is calibrated in this way, it is convenient to determine the end-point of a titration in terms of number (or fraction) of revolutions and to apply the factor to convert into volume of solution used.

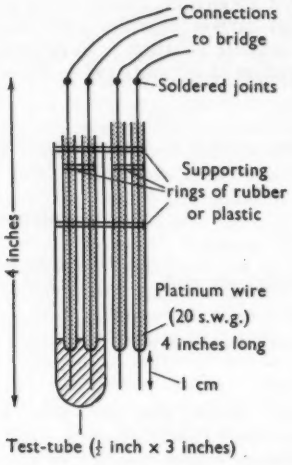

Fig. 2. Electrode assembly

METHOD

REAGENTS—

Oxygen.

Hydrogen peroxide, 100-volume—Analytical-reagent grade.

Sodium hydroxide, 0·01 N.

Indicator solutions—Dissolve 0·125 g of methyl red in 100 ml of ethanol and 0·083 g of methylene blue in another 100 ml of ethanol. For each titration, use 2 drops of each indicator solution.

Percolated ion-free water—Allow distilled water to percolate through a mixed-resin bed, and boil before use.

Ammonia solution, approximately 0·04 N.

Isopropyl alcohol—Analytical-reagent grade.

Barium acetate, 0·1 N.

Silver nitrate, approximately 0·1 N.

Hydrochloric acid, 2 per cent. w/w.

Cation-exchange resin—Amberlite IR-120(H).

PREPARATION OF SAMPLE FOR COMBUSTION—

Solids—Solids are weighed in filter-papers that have been folded lengthwise in three and stored in the balance case before use. After weighing, the sides of the paper are folded over the sample, and the paper is then folded in three to make a small square, with the fuse protruding. The enclosed sample is then supported in the bend of the platinum wire, as shown in Fig. 3 (*b*), so that the fuse is directed away from the stopper and in line with the platinum wire.

Viscous liquids—These are weighed in filter-papers that have been folded in the shape of an M and stored. The sample is placed in the depression and does not make contact with the balance pan during weighing. After weighing, the paper is enclosed in a platinum-gauze support of the type shown in Fig. 3 (*a*), with the fuse directed away from the stopper.

Less viscous liquids can be conveniently weighed by difference from a melting-point tube, the sample being transferred to a prepared filter-paper already positioned in an S-bend platinum support as shown in Fig. 3 (*b*).

Volatile liquids—These are conveniently weighed in sealed tubes made from cellulosic adhesive tape[6] as described below, although this method cannot be used if the sample dissolves the tape.

Attach a $\frac{3}{8}$-inch-square piece of filter-paper, with attached fuse, in the centre of a 1-inch-square piece of cellulosic adhesive tape as shown in Fig. 3 (*d*). Roll the square round a $\frac{3}{16}$-inch glass rod, withdraw the rod, and press one end of the tube firmly together to make a seal (the fuse will protude at the open end). Weigh the container, introduce the sample, seal the open end firmly, and re-weigh. Support the sealed tube in a platinum-gauze holder of the type shown in Fig. 3 (*a*), with the fuse protruding. (Note that an extremely volatile material may escape through the seal; for such a sample, the capsule is better used without an attached fuse. After sealing and weighing, a fuse can be inserted in the gauze support along with the capsule.)

Hygroscopic or unstable solids—These are weighed in cellulosic adhesive tape - filter-paper containers, as shown in Fig. 3 (*d*), prepared by folding the square along the middle and pressing the adhesive edges together. This is simpler than putting solid material into the capsule described above for volatile liquids.

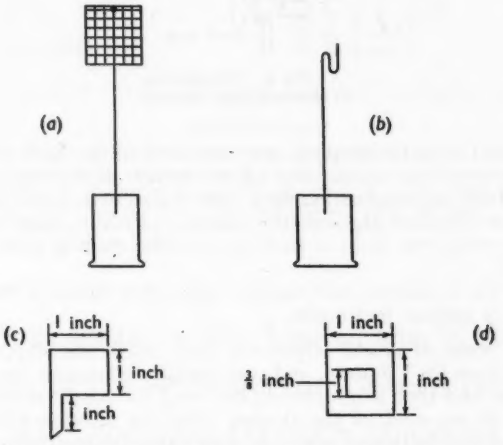

Fig. 3. Types of support for samples during combustion: (*a*) and (*b*), stoppers fitted with platinum supports; (*c*) prepared piece of filter-paper; (*d*) prepared piece of cellulosic adhesive tape - filter-paper

PREPARATION OF SOLUTION—

Compounds containing only C, H, O and S or C, H, O, S and M, where M is a metal having a water-soluble sulphate, are decomposed by combustion in a 250-ml flask containing 10 ml of 10-volume hydrogen peroxide previously neutralised with 0·01 N sodium hydroxide to methyl red - methylene blue indicator; this is solution A. Compounds containing C, H, O, S, N, Cl, Br, I, P and M are decomposed in a 250-ml flask containing 5 ml of 20-volume hydrogen peroxide; previous neutralisation is unnecessary. This is solution B.

TREATMENT OF SOLUTION AFTER COMBUSTION—

Solution A used; metal M absent—Boil the solution for 1 minute, cool rapidly, add 2 drops more of each indicator solution, and titrate with 0·01 N sodium hydroxide.

Solution A used; metal M present—Prepare a slurry of Amberlite IR-120(H) with water, fill a column, of the type shown in Fig. 4, to the base of the reservoir with water, and pour in portions of the slurry. Allow the resin to settle, and fill the column to the base of the reservoir with resin. Drain off the water until the resin is just covered. Add 20 ml of 2 per cent. hydrochloric acid, allow it to percolate dropwise through the resin, and wash the column with water until the washings are neutral to the mixed indicator used in the subsequent titration. Again drain off the water until the resin is just covered.

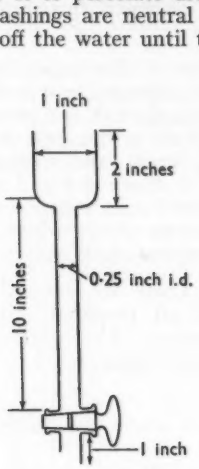

Fig. 4. Dimensions of ion-exchange column

Open the flask, and rinse the stopper, wire and neck of the flask with water. Pour the solution into the reservoir of the column, and allow it to percolate dropwise; collect the effluent in a 250-ml flask. Wash the combustion flask three times with small portions of water, and allow each washing to percolate through the column. Finally, rinse the column reservoir, and turn off the tap when the resin is just covered (the resin is then ready for the next percolation).

Boil the effluent for 1 minute, cool rapidly, add a few drops of the screened indicator, and titrate with 0·01 N sodium hydroxide.

Solution B used; metal M absent—Open the flask, wash the stopper, wire and neck of the flask; boil the solution for 1 minute, and cool rapidly. Transfer the solution to a 100-ml beaker; use water first and then isopropyl alcohol until the total volume is 50 to 60 ml and the solution contains 50 per cent. of the alcohol. Stir the solution with a magnetic stirrer, add 1 drop of methyl red indicator solution, and carefully neutralise with the ammonia solution (added from a burette supported over the beaker).

In the absence of phosphate ions, the solution is ready for titration. If phosphate ions are present, add a calculated amount of approximately 0·1 N silver nitrate; calculate the required volume from the figures below.

0·1 ml for each 0·1 mg of phosphorus present
0·1 ml for each 0·36 mg of chlorine present
0·1 ml for each 0·80 mg of bromine present

Add 0·2 ml of the silver nitrate solution in excess, and again neutralise to methyl red with the ammonia solution.

CONDUCTIMETRIC TITRATION WITH BARIUM ACETATE SOLUTION—

Immerse the electrode assembly, switch on the bridge, and obtain the balance point. The solution in the reference dip cell contains about 2 ml of water, to which 0·01 N sodium hydroxide is added until a bridge reading (ratio of test cell to reference cell) of about 0·5 is obtained. Once corrected, this solution will be suitable for many similar determinations, but the addition of more sodium hydroxide will be necessary when changing to test solutions

to which silver nitrate has been added. If a sharp balance point is not obtained, connect a suitable (0·001- to 1-μF) variable capacitance across the electrodes in the test solution.

Titrate the solution with 0·1 N barium acetate added from the syringe-burette. The magnitude of the increments will depend on the concentration of sulphate ions and should be such that at least five readings are obtained before the end-point. Take a similar number after the end-point has been passed, and obtain the end-point graphically.

Metal M present—Allow the combustion solution to percolate through the column as before, and collect the effluent in an Erlenmeyer flask. Boil for 1 minute to expel carbon dioxide (or longer if the volume of solution needs to be decreased), cool rapidly, and transfer to a 100-ml beaker with water and isopropyl alcohol. Proceed with the conductimetric titration, including the treatment with silver nitrate if phosphate ions are present.

Results

Some of the results found are shown in Table I (p. 599); others are presented in Table II.

TABLE II

SULPHUR FOUND IN COMPOUNDS CONTAINING C, H, O, S, N, Cl AND P

Sample	Elements present	Sulphur content found, %	Theoretical sulphur content, %
Sulphonal	C, H, O and S	28·0	28·1
Dibenzal disulphide	C, H, O and S	25·9	26·0
S-Benzylthiuronium chloride	C, H, N, Cl and S	15·8	15·8
Phenylthiourea	C, H, N and S	21·1	21·1
Diallylphenylphosphine sulphide	C, H, S and P	14·5	14·4

I thank the Directors of "Shell" Research Ltd. for permission to publish this paper.

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Developments in the Use of the Wide-bore Dropping-mercury Electrode for Determining Dissolved Oxygen and Oxygen in Gases

BY R. BRIGGS AND G. KNOWLES

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Improvements in the technique of using the wide-bore electrode include its adaptation for measuring the dissolved-oxygen contents of samples in bottles and samples flowing past the electrode at rates between 1 ml and several litres per minute. A reagent solution recommended contains sodium starch glycollate as a new type of maximum suppressor. Three types of reference electrode are suitable; one is a silver rod coated with silver chloride and is directly immersed in the sample. The determination of oxygen in gases is also described.

SINCE the appearance of a previous paper on the wide-bore dropping-mercury electrode,[1] experience has been gained in its use for indicating and recording the concentration of dissolved oxygen in water and the oxygen content of gas. Other workers[2,3] have used the electrode for recording contents of dissolved oxygen, and it is available commercially for this purpose and for general polarography.

FORMS OF CELLS AND ELECTRODES AND THEIR APPLICATIONS

DOWNWARD-POINTING ASSEMBLY (WITH UPTURNED ELECTRODE)—

An assembly suitable for measuring the dissolved-oxygen contents of samples in bottles is shown in Fig. 1 (*a*); we are grateful to Dr. H. Clay for suggesting use of the wide-bore electrode for this purpose. With this arrangement, a complete dissolved-oxygen indicator can be built in a portable box; the pointer-type meter can be graduated directly in parts per million of dissolved oxygen, and the range can be from 0 to 5 or up to from 0 to 50 p.p.m., with range switching if desired.

With this assembly, it is essential for the connections to the thermistors to be thoroughly water-proofed. If the wires become wet, errors are caused, not so much by partial short-circuiting of the thermistors as by the presence in the circuit of local cells formed by the copper wires of one thermistor and the solder on the connections to the other. By using a plastic sealing compound (as manufactured by W. T. Henley's Telegraph Works) and a covering sleeve of silicone-elastomer tubing, good water-proofing can be achieved; the details of this method have been described by Briggs and Mason.[4]

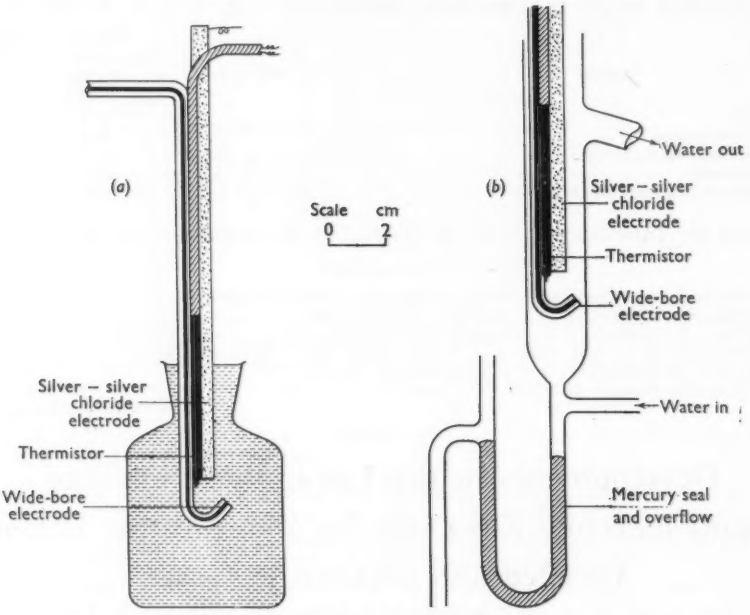

Fig. 1. Downward-pointing electrode assembly for measuring dissolved oxygen: (*a*) in a bottle; (*b*) in a flowing liquid

The downward-pointing assembly can also be used, as shown in Fig. 1 (*b*), for samples flowing past the electrode. The readings remain unchanged at any flow up to 30 ml per minute and increase by 3 per cent. at 70 ml per minute (linear velocity 0·35 cm per second). The effect of flow is minimal when the relative directions between electrode and flow are as shown in Fig. 1 (*b*).

OTHER ARRANGEMENTS OF CELL AND ELECTRODE—

The cell shown in Fig. 2 permits a flow of several hundred millilitres of sample per minute past the electrode for an increase of 3 per cent. in the polarographic current. In practice, however, the cell has generally been used for readings on stationary samples pumped automatically for 1 minute through the cell every 30 minutes; an electromagnetic valve then clamps the flexible inlet tube to hold the sample in the cell for 2 minutes, during which the reading is made and recorded on a strip-chart. For automatic recording on rivers, away

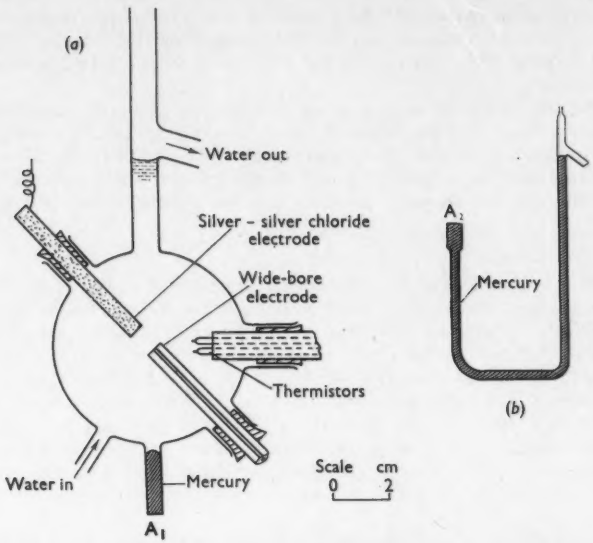

Fig. 2. Large-volume cell for recording dissolved-oxygen content: (*a*) cell; (*b*) mercury seal and overflow. A_2 is attached to A_1 by poly(vinyl chloride) tubing

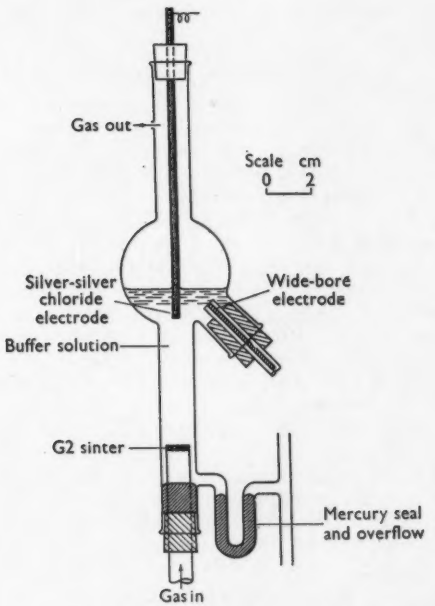

Fig. 3. Cell for recording oxygen content of a gas (lines indicate internal surfaces)

from mains power, this minimises the size of accumulator needed and also facilitates the addition of reagent (described later); the solution is introduced by means of a simple electromagnetic valve system into the surface of the sample in the cell just after pumping has ceased, there then being sufficient movement remaining in the water to ensure that reagent is mixed with sample.

For recording the concentration of dissolved oxygen in small volumes of water flowing at about 1 ml per minute, Edwards and Learner[3] used a small cell containing a wide-bore electrode and a mercury-pool reference electrode. Good stability of calibration was shown on a recorder chart reproduced in their paper; readings were within about 0·05 p.p.m. of the true value, and the calibration was the same for any rate of flow between 1·0 and 2·6 ml per minute.

RECORDER FOR OXYGEN CONTENT OF GASES—

Fig. 3 shows a cell fitted with a wide-bore electrode for automatically recording the oxygen content of a gas, the method being to equilibrate the gas under examination with a buffer solution (25 g of hydrated disodium hydrogen orthophosphate and 4 g of potassium chloride dissolved in 1 litre of distilled water) and then to record the concentration of dissolved oxygen in the solution; to saturate the solution, the gas is bubbled through it at 50 ml per minute for 2 minutes. The same principle was used by Wise,[5] with a conventional dropping-mercury electrode, but he did not report the stability of calibration. Our instrument has retained its calibration during a year of continuous operation, 24 hours a day, and is arranged to record alternately the oxygen contents of the atmosphere (as a check) and of air that has passed through the liquor in a continuous-culture vessel; the complete recording cycle takes 8 minutes.

Because the solubility of oxygen decreases with increasing temperature at almost the same rate as the polarographic current increases, compensation for variations in temperature of the buffer solution is unnecessary, provided that the gas is brought to about the same temperature as the solution before being bubbled through it. A change in barometric pressure affects the reading, but the error is small and can be corrected for if the barometric pressure is known. Alternatively, the trace for "oxygen in air" can be rapidly brought back to the "21 per cent." position on the chart if it has moved from this value because of a change in barometric pressure.

PREPARATION OF CAPILLARIES

The length of 0·2-mm bore capillary is about 30 cm when the vertical distance between the level of the mercury supply and the delivery tip of the wide-bore electrode is 15 cm; this gives a drop-time of about 3 seconds. The 0·8-mm bore capillary may be of any length.

After the 0·8-mm capillary has been bent to its final shape it is best to apply a coating of silicone, hard baked on to its interior, especially if the electrode is to be used in concentrated saline solutions. The capillary is washed through with ethyl methyl ketone and dried by passing dry dust-free air through it for 2 hours. Next, it is left full of dilute hydrochloric acid (1 + 9) for 24 hours, washed, first with water and secondly with ethyl methyl ketone, and again dried as before. It is then washed with a solution of 3 ml of MS1107 Silicone Fluid (Midland Silicones Ltd.) in 100 ml of ethyl methyl ketone and dried in air for 4 hours. Finally, the capillary is placed in an oven at 110° C for at least 12 hours and is then cooled, washed through with ethyl methyl ketone and dried in air for 2 hours.

The 0·2-mm capillary is not coated with silicone, although it should be washed through with ethyl methyl ketone and dried by passage of air. If the ends of this capillary are "flame-polished," care should be taken not to constrict the internal diameter, or air bubbles will be difficult to remove when the tube is first filled with mercury.

When used in water containing calcium bicarbonate, a deposit of calcium carbonate slowly forms at the orifice of the wide-bore electrode (unless the determinations include addition of the reagent solution). The deposit can easily be removed by successively washing the capillary through with concentrated hydrochloric acid, water and ethyl methyl ketone and then drying in air.

REFERENCE ELECTRODES

The reference electrode described previously[1] consisted of a zinc rod in an acetate buffer solution; it is satisfactory, but requires weekly servicing of the zinc rod and glass sinter and weekly replacement of the buffer solution. Subsequently, an electrode consisting of a

silver rod coated with silver chloride and directly immersed in the sample has been found satisfactory and does not require servicing. It is made from "fine silver" rod ($\frac{1}{8}$ or $\frac{1}{4}$ inch in diameter), which is cleaned with ethyl methyl ketone, washed with water, immersed for 1 minute in diluted nitric acid (1 + 1) and is then made the anode in 0·1 N hydrochloric acid, the other electrode being conveniently a platinum wire; after a current of about 1 mA has been passed for about 1 hour the electrode is ready for use. According to Lingane,[6] a silver - silver chloride electrode directly immersed in the sample can be used for any sample that gives at least a faint opalescence when 2 drops of 0·1 N silver nitrate are added to 10 ml of it. For measuring dissolved oxygen by means of the wide-bore electrode in conjunction with the silver - silver chloride electrode, an applied voltage of −1·5 volts (mercury negative) is suitable. When the lay-out of the cell permits, a pool of mercury may be used as reference electrode, as was done by Edwards and Learner.[3]

Reagent solution

When the equipment is to be used with a wide variety of samples, it is advantageous to make a routine addition of a multi-purpose reagent solution to each sample; this solution is prepared as described below.

Take 2500 ml of a solution containing 55 g of sodium starch glycollate per litre. (This is obtainable as a solution at this concentration from the British Drug Houses Ltd. or can be made from the solid by allowing this to soak in the water overnight and then applying heat, with stirring, to complete solution; both at this stage and subsequently, the solution should not be heated to a temperature in excess of 50° C.) In the solution dissolve, in this order, 45 g of sodium hexametaphosphate, 670 g of sodium chloride, 190 g of potassium carbonate, 80 g of potassium chloride, 220 g of potassium nitrate and 130 g of glycine; laboratory-grade reagents are satisfactory. Make up to 3000 ml with distilled water.

This reagent solution is added in the proportion of 0·5 ml per 100 ml of sample.

The advantages of this procedure are—

(*i*) The conductivity of the sample may be too low; for example, a conductivity of less than 200 micromho per cm may, in conjuction with a concentration of dissolved oxygen about that given by saturation with air, result in appreciable loss of voltage across the resistance of the solution, thereby decreasing the accuracy. The reagent solution increases the conductivity of the sample to at least 3000 micromho per cm.

(*ii*) Although marked maxima do not seem to occur on the second plateau of oxygen reduction, they can cause some error, but are entirely eliminated by the sodium starch glycollate in the reagent solution.

(*iii*) The pH of the sample may be less than 7, which may allow interfering metals to remain in solution, or less than 4·5, which may result in additional current due to reduction of hydrogen ions; alternatively, it may be above 9, and this, in conjunction with lack of buffering, increases the tendency of calcium carbonate to be deposited on the electrode. Addition of the reagent solution brings the pH to about 8 and increases the buffering capacity; further, it guards against deposition of calcium carbonate, as the reagent solution contains sodium hexametaphosphate.

Avoidance of chemical interference

Perhaps because of their readiness to be precipitated, metals do not interfere with the determination of dissolved oxygen by means of the wide-bore electrode if the pH is 6·5 or more. The metals tested were copper (Cu^{2+}), cadmium, chromium (Cr^{3+}), lead, tin (Sn^{2+}), nickel (Ni^{2+}), iron (Fe^{3+}) and zinc, added in the ionic form, each at a concentration of 5 p.p.m., singly or as a mixture. This absence of interference was also found when the reagent solution was added, the pH then being brought to about 8 by the buffering action of the reagent solution itself.

The ions Fe^{2+} and Mn^{2+} are special cases, because a pH of more than 6·5, as with the other metals, causes them to be precipitated as the hydroxides; these hydroxides have no direct effect on the polarographic determination of dissolved oxygen, but, when precipitated, remove an equivalent amount of dissolved oxygen.

No effect on the determination of dissolved oxygen when using the second oxygen plateau was produced by the presence of 250 p.p.m. of nitrite (expressed as NO_2^-), 144 p.p.m. of

sulphide added as Na_2S (concentration expressed as S^{2-}) or 40 p.p.m. of ammonia (expressed as N), either when these were added singly or all together, and no effect was produced by 60 p.p.m. of sulphite (expressed as SO_3^{2-}). The silver - silver chloride electrode was not affected by these concentrations of nitrite, sulphide, ammonia and sulphite.

Mercury-level control and temperature compensation

For recorders, automatic maintenance of the level of the supply of mercury is necessary, and, in addition to the electrical method, a non-electrical technique has proved reliable; a rod, attached to the top of a float in the mercury level to be controlled, presses against a compressible tube leading from the mercury-supply reservoir.

Automatic compensation for the temperature of the sample by means of thermistors was described earlier,[1] and Littlewood[7] has devised an alternative method of calculating the values of the resistances used in the circuit.

Assistance has been given by P. J. Lee and W. H. Mason. This paper is published by permission of the Department of Scientific and Industrial Research.

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Amended, *April 28th*, 1961

Recommended Methods of Analysis of Pesticide Residues in Foodstuffs

REPORT BY THE JOINT MERCURY RESIDUES PANEL

Set up jointly by the Scientific Sub-Committee of the Advisory Committee on Poisonous Substances Used in Agriculture and Food Storage, the Analytical Methods Committee of the Society for Analytical Chemistry, and the Association of British Manufacturers of Agricultural Chemicals

The Determination of Mercury Residues in Apples and Tomatoes

In 1959, the Scientific Sub-Committee of the Advisory Committee on Poisonous Substances Used in Agriculture and Food Storage, the Association of British Manufacturers of Agricultural Chemicals and the Analytical Methods Committee of The Society for Analytical Chemistry set up a Panel to investigate the analysis of residues of mercury-containing pesticides in foodstuffs. Subsequently, the Food Manufacturers Federation Incorporated accepted an invitation to join in the work of the Panel. A collaborative study was undertaken with the object of recommending a detailed method that would give accurate and reproducible results. This report describes the findings of the Panel and gives details of a method suitable for determining mercury in apples and tomatoes.

Members of the Panel are listed in Appendix II.

Course of the investigation

A method (unpublished) for determining mercury residues in apples had been developed in the Department of Scientific and Industrial Research, Laboratory of the Government Chemist (then the Department of the Government Chemist), on the basis of Klein's method as modified by Abbott and Johnson.[1] This method (Department of the Government Chemist Report No. GC/17/17) has been found reliable by workers of that laboratory for residues of a wide range of organo-mercury compounds. In outline, it consists in digesting the

sample with concentrated nitric and sulphuric acids and with hydrogen peroxide, extraction of the partly neutralised digest with dithizone in chloroform and measurement of the colour of the mercury - dithizone complex at 490 mμ after employing a thiosulphate reversion technique to remove cations interfering in the determination. The Panel gave attention to suggestions for modifying this outline. It also noted that two special methods for the wet decomposition of organic matter for the determination of mercury have recently been published by the Analytical Methods Committee of the Society for Analytical Chemistry.[2] The first of these is applicable to samples containing high mercury residues (>5 p.p.m. or, alternatively, >5 mg per kg) and the second is for lower mercury levels in urine or animal tissue. The method used in this study has been considered in relation to low mercury residues in vegetable material.

Phenylmercuric acetate was used as a convenient organo-mercury compound in these studies, since it had been shown to be more volatile than others of its type used as fungicidal sprays and aerosols.[3] When added to diced apple and digested with acids, phenylmercuric acetate gave a lower recovery than did the salicylate or the nitrate. The Panel considered that a satisfactory method for phenylmercuric acetate would be satisfactory in general.

TABLE I

MEAN BLANK VALUES OF MERCURY FOR REAGENTS AND FRUIT

Laboratory	Mercury found in— reagents, μg	50 g of apple (less reagent blank), μg	50 g of tomato (less reagent blank), μg
1	0·7	0·4	0·3
2	0·3	0·2	0·3
3	1·7	nil	nil
4	1·3	nil	0·3
5	3·4	1·5	0·9
6	0·6	0·6	0·3
7	1·2	0·3	0·2
8	1·2	0·1	nil
9	0·1	0·2	0·4
10	2·5	—	0·1

Laboratories had been advised to purchase small amounts of dithizone at intervals of about 3 months, because of the instability of the reagent. Material purchased in this way did not always ensure a satisfactory standard solution, and instructions for the purification of the dithizone[4] were inserted in the method. Chloroform was purified according to the method of the Analytical Methods Committee.[5] Correspondence with firms supplying sodium hypochlorite solutions revealed that these solutions had a variable and sometimes undesirably high mercury content. Messrs. Hopkin and Williams Ltd.* supply a "low in mercury" grade of 10 per cent. w/w sodium hypochlorite solution containing amounts of mercury of the order of 0·12 p.p.m. and state that it is known to be suitable for use in the determination of mercury as described by Abbott and Johnson.[1] Some selenium samples also showed an undesirably high mercury content, and levels of 12, 16 and more than 50 p.p.m. have been found. Even with these precautions, some laboratories obtained high reagent values (Table I). Although the blanks varied from laboratory to laboratory they were fairly consistent in any one laboratory. The Panel considered it desirable to keep reagent blanks to about 1 μg or below.

The Panel used method GC/17/17 initially for the recovery of 5 μg of mercury added to 50 g of apples (0·1 p.p.m.). However, both the blank values obtained for the apples and the recovery of added mercury were variable (see Table II, column A), due perhaps to each laboratory using its own sample of apples. A common apple sample in the form of a purée was circulated for the second study. Satisfactory blank values and recoveries were obtained with this. Net recoveries are set out in Table II, column B. It was noted, however, that since a part of the skin and core of the apples had been separated during the preparation of the purée the digestion stage proceeded rather easily. The need for careful digestion of the pulp with the acids was stressed, together with the need to allow sufficient time for the hydroxylamine hydrochloride solution to react with the residual oxidising material before extracting the partly neutralised digest with dithizone.

* Freshwater Road, Chadwell Heath, Essex.

Next, small glass ampoules containing a known amount of phenylmercuric acetate solution were circulated to members of the Panel, to use in conjunction with samples of canned purée made from whole apples. In this way an amount of mercury (3·4 μg) unknown to the worker was added and recovered. The results (see Table II, column C) show a mean net recovery of 80 per cent. with a standard deviation of ±0·4 μg. The Panel concluded that the method was satisfactory for determining mercury residues in apples.

TABLE II

NET RECOVERY OF PHENYLMERCURIC ACETATE ADDED TO APPLE

	Net recovery of—		
Laboratory	5 μg of mercury (known) from members' own apple sample, A, μg	5 μg of mercury (known) from apple purée less some skin and cores, B, μg	3·4 μg of mercury (unknown) from purée of whole apple, C, μg
1	5·1, 4·9	4·6, 4·7, 5·1, 5·5	2·5, 2·5, 3·0, 2·8, 2·4
2	4·7, 4·3, 4·0, 4·4	4·8, 4·9, 4·8	2·8, 2·6, 2·5, 2·7, 2·9, 2·5
3	3·1, 3·3, 1·3, 5·2, 3·2	4·4, 4·2, 4·4	3·0, 3·3, 3·0
4	3·2, 4·7, 3·3, 4·3, 4·6	4·5, 4·6, 4·8	3·0, 2·5, 2·4, 2·9
5	4·4, 4·4, 4·7	—	—
6	4·6, 5·1, 4·8	4·5, 4·3, 4·6	2·3, 2·9, 2·5, 2·3
7	4·5, 4·4, 4·8, 4·7, 4·6, 4·3, 4·5, 4·3, 4·3, 4·1	4·6, 4·4, 4·4	2·5, 2·9, 1·9
8	3·4, 3·0, 4·7	5·2, 5·0, 6·0, 5·4, 5·2	2·0, 2·5, 2·2, 2·0, 2·2
9	—	4·2, 4·5, 4·1	2·8, 2·8, 2·6, 3·1
Mean	4·2	4·7	2·6
S.D.	0·8	0·5	0·4

A similar approach was adopted in recovery experiments with a sample of canned tomato purée prepared from the whole fruit. A mean net recovery of 80 per cent. with a standard deviation of ±0·6 μg was obtained when 4·0 μg of mercury (0·08 p.p.m.) were added to the purée and a mean net recovery of 95 per cent. with a standard deviation of ±0·9 μg when 20·1 μg (0·40 p.p.m.) were added (see Table III). The Panel concluded, therefore, that the method was also satisfactory for determining mercury residues in tomatoes. The experience of some members suggested that selenium might be omitted from the method for tomatoes at lower levels of mercury.

The method is given in Appendix I. Limited experience of some members indicates that the method should be satisfactory with other plant materials, but recovery experiments are regarded as a necessary preliminary. When the water content of the material is low it is necessary to add water (5 to 10 ml per 50 g of sample) to the contents of the digestion flask initially. Additionally, limited experience suggests that the method is successful in the presence of arsenic and lead with apples to which Mercurated Lead Arsenate had been added.

The Panel also concluded that the method required experienced workers. Carefully screened reagents should be used in a laboratory set aside from work involving macro amounts of mercury compounds.

TABLE III

NET RECOVERY OF PHENYLMERCURIC ACETATE ADDED TO TOMATO

Laboratory	Net recovery of— 4·0 μg of mercury (unknown), (no selenium), μg	4·0 μg of mercury (unknown), μg	20·1 μg of mercury (unknown), μg
1	3·5, 3·5, 3·4, 3·4, 3·4	3·4, 3·6	18·2, 18·1, 18·0, 17·6
2	3·7, 3·6, 3·8, 3·4, 3·9, 3·6	—	18·9, 19·4, 19·5, 19·6, 18·8, 19·2
3	3·7, 4·0	—	19·5, 19·7
4	3·1, 3·1, 3·0, 3·3	—	18·7, 19·3, 19·0, 19·1
5	1·6, 3·1, 1·7, 4·0	—	18·6, 17·4, 18·8, 20·8
6	3·2, 3·2, 3·2	—	19·6, 18·3, 18·7
7	3·3, 3·4, 3·4	3·3, 3·4, 3·3	17·5, 18·9, 17·5
8	2·6, 2·6, 2·8, 2·8, 2·5, 2·4	—	20, 20, 20·5, 20, 19·5, 20·4
9	3·1, 3·3, 3·8	—	20·5, 19·5
Mean	3·2	—	19·1
S.D.	0·6	—	0·9

Appendix I

RECOMMENDED METHOD FOR DETERMINING MERCURY RESIDUES IN APPLES AND TOMATOES

APPARATUS—

All apparatus must be scrupulously cleaned. It is recommended that, after assembling the digestion apparatus, a sulphuric - nitric acid mixture (30 ml + 20 ml) be boiled under reflux in it.

Round-bottomed flask (500 *ml*), with two short necks carrying B34 (central) and B19 (side) sockets.

Air condenser, about 2·5 cm diameter, 30 cm long, B34 cone and B24 socket.

Water condenser—This should be efficient; 15-cm double surface or 25-cm jacketed spiral has been found adequate. The water condenser fits into the top of the air condenser and should have a B24 cone.

Heating mantle, 250 *watt* (500 *ml*), with variable heat control (preferably continuous).

Spectrophotometer.

Tap funnel—B19 cone.

REAGENTS—

Reagents should be of analytical grade except when stated otherwise.

Water—Redistil from all-glass apparatus.

Selenium powder—Select for low mercury content.

Sulphuric acid, sp.gr. 1·84.

Nitric acid, sp.gr. 1·42.

Hydrogen peroxide, 100-*volume*.

Chloroform, B.P., purified.[5]

Ammonia, sp.gr. 0·880.

Acetic acid—Dilute to 30 per cent. v/v.

Hydrochloric acid, about 0·1 N—Dilute 50 ml of acid, sp.gr. 1·18, to 5 litres.

Sodium hydroxide, about 0·1 N—Dissolve 20 g of general reagent grade sodium hydroxide in 5 litres of water.

Sodium thiosulphate solution—Weigh 1·5-g portions in clean sample tubes, and dissolve in 100 ml of water to give fresh solutions each day.

Sodium hypochlorite solution—Select 10 per cent. w/w sodium hypochlorite solution with a low mercury content. Store at about 3° C when not in use. Standardise with sodium thiosulphate, and dilute to about 4·5 per cent. of available chlorine.

Hydroxylamine hydrochloride solution, 20 per cent. w/v, aqueous—Extract with stock dithizone solution until the reagent colour is unchanged, then wash thoroughly with chloroform until three successive washes are colourless.

Dithizone solution, purified[4]—Prepare (*i*) a stock solution of 200 mg per litre of chloroform (this should be stored at about 3° C) and (*ii*) two dilute solutions of 4 mg per litre of chloroform for extraction and as a standard solution.

Mercuric chloride solution—(*i*) Stock solution of 1·355 g of mercuric chloride in 1 litre of 0·1 N hydrochloric acid; this solution is stable for at least 6 months. (*ii*) Dilute solution, freshly prepared by diluting the stock solution in two stages with hydrochloric acid to a concentration of 1 μg of mercury per ml; dilute mercuric chloride solutions are stable for at least 24 hours in dilute acid, but not in neutral solutions.

Cotton-wool, BPC.

DITHIZONE - MERCURY CALIBRATION CURVE—

By pipette, place 1 to 5 ml of freshly diluted mercuric chloride solution (1 μg per ml) in a separating funnel containing 50 ml of hydrochloric acid, 5 ml of hydroxylamine hydrochloride and 3 ml of acetic acid solutions. Add 2 to 3 ml of chloroform, and shake the funnel vigorously for 30 seconds. Allow the layers to separate, and reject the chloroform. Extract the mercury with 10·0 ml of standard dithizone solution, freshly diluted from the stock solution, shaking vigorously for 1 minute. Dry the stem of the separating funnel with a segment of filter-paper, fit a loose plug of cotton-wool in the lower part of the stem, allow a little of the dithizone extract to run to waste through the cotton-wool plug, and run the remainder into the spectrophotometer cell. Care should be taken to ensure that the cotton-wool plug is not contaminated while placing it in the stem of the funnel and also that it is not dislodged by the flow of chloroform solution. Avoid dripping very slowly, or a red colour may develop; also avoid too rapid a flow, which could result in failure to trap entrained water. Wipe the optical faces of the cell, and place carefully in the cell basket. Read the optical density at 490 mμ against chloroform. A Unicam 4-cm cell requires about 8 ml of solution for a satisfactory reading.

The undesirably large background absorption of dithizone solution, occupying the most sensitive part of the scale, may be suppressed by replacing the chloroform reference cell with a coarse grating of, for example, 29/30 s.w.g. (0·345 to 0·315 mm) woven copper gauze, which is firmly supported in the cell basket. The zero on the calibration curve can thus be reduced to an optical density of about 0·1.

With phosgene-free chloroform, the dilute dithizone solutions may be used for a few weeks. The zero value should be checked daily before measuring the extracted solution and, if necessary, a small correction applied to the optical density of the latter before reading its corresponding mercury content on the calibration curve. Discard any dithizone solution for which the zero value has fallen markedly with time or when about 90 per cent. has been used, at which time the risk of autoxidation is greater. Solutions of dithizone should be stored in the dark; dilute solutions of dithizone fade in 30 seconds when exposed to bright sunlight.

DIGESTION OF THE FRUIT SAMPLE—

Macerate a sample of tomatoes, and take a 50-g portion for analysis; cut apples into quarters or segments, and use a diced 50-g portion. Place the prepared sample in the reaction flask together with a few glass beads, and mix with 0·1 g of selenium powder. Seat the flask in the heating mantle, and fit the condenser system (with water flowing rapidly through it) and the tap funnel. Add carefully from the tap funnel over about 10 minutes 20 ml of sulphuric - nitric acid (3 + 1), taking care that the reaction at no time becomes violent. The reaction mixture simmers from its exothermic heat, the mantle helping to lag the flask. When the initial reaction has subsided, switch on the heating mantle and slowly increase

the rate of heating over ½ to ¾ of an hour as frothing in the flask abates. It may be necessary to add further small amounts of nitric acid to prevent carbonisation, which can occur quite abruptly. Up to 20 ml of nitric acid may be required, depending on the species and moisture content of the sample. When at "Full Heat," leave the digest to boil under reflux for 3 hours. At the end of this period the apple digest should appear a darkish green and homogeneous; the tomato digest is yellow. Cool, and add 10 ml of hydrogen peroxide from the tap funnel. Boil under reflux for 1 hour. Cool the digest (now a pale green for apples), and wash down the condenser with 50 ml of water.

This is the only stage at which, if it is necessary, the process may be interrupted.

Filter the cold digest through a Whatman No. 541 filter-paper into a graduated vessel, and dilute to 250 ml. Transfer to a 500-ml separating funnel, rinse the graduated vessel with (exactly) 50 ml of water, and transfer the washings to the separating funnel. Titrate a 1-ml aliquot of the 300 ml (now about 2·5 N) with 0·1 N sodium hydroxide (= S ml) with methyl red as indicator, and adjust the acidity of the digest to normal with ammonia solution (= x N).

$$\text{Volume of ammonia to be added} = 300\ (S/10 - 1)/(x + 1)\ \text{ml}.$$

EXTRACTION OF MERCURY FROM THE DIGEST—

Cool the solution thoroughly before adding 5 ml of hydroxylamine hydrochloride solution to avoid dithizone being oxidised or selenium being precipitated. Set aside for 10 minutes to allow the hydroxylamine to react, and then add 10 ml of dilute dithizone extraction solution. Shake gently, and release the pressure cautiously; then insert the stopper tightly, and shake the funnel vigorously for 1 minute. Allow the two layers to separate, and examine the colour of the chloroform layer. Shake for a further minute, and re-assess the colour; there are three situations—

(1) The first colour is green or grey, and is not changed markedly on subsequent shaking. Transfer the chloroform phase to a 150-ml separating funnel containing 25 ml of hydrochloric acid and 5 ml of hydroxylamine hydrochloride solution. Extract the initial aqueous solution twice more with 5 ml of dithizone solution, shaking the funnel for 1 minute, and transfer each extract to the 150-ml separating funnel. Shake the latter vigorously for 1 minute.

(2) The first colour is grey changing to yellowish orange. This suggests the oxidation of unreacted dithizone. Transfer the chloroform solution to a 150-ml separating funnel, and extract the initial aqueous solution with 10 ml of dithizone solution. This time the colour, green or possibly grey, should be more permanent. Repeated fading of the dithizone extraction solution indicates that an excessive amount of oxidant is present. Make sure the solution is cool, and try adding more hydroxylamine hydrochloride. Finish with two further extractions as in (1).

(3) An orange colour is formed immediately. This indicates a large mercury concentration. Transfer the chloroform solution to a 150-ml separating funnel, and extract the digest solution with a further 10 ml of dithizone. A grey colour suggests that the bulk of the mercury has been removed, and the extraction is finished as in (1). If an intense orange colour is formed again, more concentrated dithizone extracting solutions must be used; most conveniently, add 1 to 2 ml of the stock dithizone solution to 8 ml of chloroform. Complete as in (1).

The extraction should not be considered complete until two successive extracts retain the blue-green colour of the reagent. It is useful to note the amount of dithizone used in the extraction, since, by discounting the final two 5-ml portions and assuming an efficiency of 80 per cent. for extraction from normal sulphuric acid, the approximate mercury content and hence the appropriate range in the combinations of volume and concentration of dithizone solution and size of cell (see Table IV) can be deduced.

Transfer the combined dithizone solution extracts to a third 150-ml separating funnel containing 50 ml of hydrochloric acid and 2 ml of sodium thiosulphate solution. Immediately shake the separating funnel vigorously for 1 minute, and discard the chloroform layer; mercury dithizonate is thus dissociated and reverts to the aqueous layer, whereas copper dithizonate remains in the chloroform layer. Much of the yellow nitrated organic matter has now been removed from the mercury-containing solution, and the subsequent hypochlorite treatment will remove the rest. Shake the hydrochloric acid washings that were left in the

second separating funnel with a few millilitres of chloroform to collect the small fraction of adduct distributed in the aqueous layer, and add the chloroform to the contents of the third separating funnel. Shake vigorously, and discard all remaining drops of chloroform. If the original extract had been left standing for some time with hydroxylamine hydrochloride and hydrochloric acid, excess of reagent may have been oxidised; should the colour suggest this, extract the hydrochloric acid washings with 2 to 3 ml of dilute dithizone reagent, and then shake in the third separating funnel before washing the aqueous solutions with chloroform. Add 4 ml of diluted sodium hypochlorite solution. Give the separating funnel a preliminary swirl to release some of the chlorine, and then insert the stopper tightly. Shake, carefully at first and then vigorously for 1 minute, cautiously releasing the pressure through the tap from time to time. Little or no chlorine should now be evolved, although an excess should be present. Invert the separating funnel, open the tap, and rinse the stem with a little water. Add 5 ml of hydroxylamine hydrochloride solution, ensuring that the sides of the stopper and the neck of the separating funnel are thoroughly wetted with the reagent, and shake vigorously for 1 minute. This treatment reduces unreacted hypochlorite and residual chlorine. Blow off any gases evolved, and shake the funnel again. Wash the aqueous solution twice by vigorous shaking with a few millilitres of chloroform, and reject both washings. The first washing sometimes appears blue, but the second should be colourless. Ensure that no bubble of chloroform has been retained above the tap of the separating funnel. Add 3 ml of acetic acid.

DETERMINATION—

By pipette, place the appropriate volume (see Table IV) of standard dithizone solution in the separating funnel, and shake vigorously for 1 minute. Proceed as described under "Dithizone - Mercury Calibration Curve." Adjust the observed value of optical density for any zero correction, and determine the concentration of mercury from the calibration curve. Correct this value by subtracting the reagent blank value, so obtaining the mercury content of the sample.

TABLE IV

CELL SIZE AND CONCENTRATION AND VOLUME OF DITHIZONE SOLUTION TO BE USED

Mercury range, μg	Dithizone concentration, μg per ml	Volume of dithizone solution, ml	Cell, cm	Optical density at 490 mμ*	Maximum satisfactory optical density at 490 mμ*	Optical density per μg of mercury
0 to 10	4	10	4	0·08	1·05	0·097
0 to 40	8	20	2	0·08	1·05	0·0243
0 to 100	16	25	1	0·08	1·05	0·0097

* Assuming the optical density of the gauze to be 0·4.

Appendix II

MEMBERSHIP OF THE PANEL

The Panel consisted of E. W. Atkins, A. S. Beidas, T. E. Burke, H. Crossley, H. Egan, P. W. Lloyd, E. J. Miller, H. E. Monk (Chairman), J. A. Pickard, N. A. Smart (Secretary) and S. H. Yuen.

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Notes

THE CHARACTERISATION OF AMINES

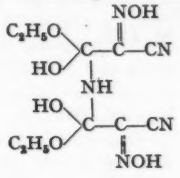
MÜLLER[1] reported that aniline or *p*-toluidine reacted with ethyl oximinocyanoacetate in ether solution to give a crystalline derivative. The structure of the derivative corresponded to the union of one molecule of the base with two molecules of the ester, and it was formed under conditions of fairly wide divergence in the proportions of the two reactants. This reaction has now been found to apply to a large number of amines, and, except for some aromatic primary amines and isopropylamine, no substance used has given negative results.

Müller, partly as a result of cryoscopic measurements, put forward the view that the compounds were loosely bound additive complexes, which dissociated completely in solution. He was able to regenerate the base and its hydrochloride by the action of potassium hydroxide and hydrochloric acid, respectively. Our findings have confirmed this view and indicate that a molecular compound is formed by the addition of a molecule of ethyl oximinocyanoacetate to a molecule of the base salt of ethyl oximinocyanoacetate.

When an aqueous solution of the aniline complex is passed through a column of ion-exchange resin (Amberlite IRA-400 in the hydroxy form), the oximinocyanoacetate is retained, and the aniline can be recovered quantitatively from the effluent. Again, treatment of the complex with excess of picric acid results in the formation of the base picrate. When the ammonium salt of ethyl oximinocyanoacetate in aqueous solution is treated with 1 mole of the ester, the same crystalline complex as that obtained directly by the action of 1 mole of ammonia on 2 moles of ester is obtained. Further, the ultra-violet absorption spectra of the derivatives show peaks corresponding to both the undissociated and the ionised forms of ethyl oximinocyanoacetate.

Further evidence for the structure of the complex derivatives is provided by the action of, for example, benzylamine on ethyl cyanoacetate or ethyl oximinocyanoacetate ethyl ether. In both reactions the amides are formed by loss of ethanol, thereby showing the necessity for the presence of the acidic oximino group for formation of the complex. Oximinocyanoacetamide when allowed to react with benzylamine forms only the benzylamine salt, which indicates that some kind of electrostatic or hydrogen bonding with the ester group is involved in formation of the complex.

The compound (melting-point 153·5° C) prepared by Diels and Borgwardt[2] by the action of nitrous acid on ethanolic malononitrile and formulated by them as (I) is doubtless the same substance as is obtained by the direct action of ammonia on ethyl oximinocyanoacetate, and it seems likely that this substance has a structure analogous to those of the other complex derivatives isolated and in keeping with the view expressed above.



(I)

It has been found that certain of the complex derivatives decompose after long standing or more rapidly when heated *in vacuo* at temperatures just below the melting-point. However, unlike Müller,[1] we found that all the compounds prepared were sufficiently stable to withstand the recrystallisation necessary for their purification. For purposes of characterisation, these derivatives have the advantage over picrates that they melt without decomposition, and the spread of temperatures for chemically related amines appears to be satisfactory.

METHOD AND RESULTS

A wide range of amines, including heterocyclic compounds, has been found to react readily with ethyl oximinocyanoacetate; the procedure used is described below.

A warm aqueous or aqueous ethanolic solution of the amine (1 mole) is added to a warm aqueous solution of ethyl oximinocyanoacetate (2 moles; prepared in the usual way from ethyl cyanoacetate), and the mixture is set aside for a few minutes. When cool, the crystalline product is collected and recrystallised from water, benzene or another suitable solvent; two recrystallisations at most are necessary.

The results for several typical samples are shown in Table I. Of the compounds tested only aromatic amines (other than aniline and *p*-toluidine) and isopropylamine failed to give crystalline products.

TABLE I

RESULTS FOR DERIVATIVES OF VARIOUS AMINES

Amine	Melting-point of derivative, °C	Formula of derivative	Carbon content found, %	Hydrogen content found, %	Theoretical carbon content, %	Theoretical hydrogen content, %
Ammonia[2]	154[a]	$C_{10}H_{15}N_5O_6$	39·7	5·2	39·9	5·0
Methylamine	160[a]	$C_{11}H_{17}N_5O_6$	42·2	5·6	41·9	5·4
Ethylamine	99[a]	$C_{12}H_{19}N_5O_6$	43·7	6·0	43·8	5·8
Ethylenediamine	162[a]	$C_{22}H_{32}N_{10}O_{12}$	41·7	5·3	42·0	5·1
Benzylamine	159[a]	$C_{17}H_{21}N_5O_6$	52·2	5·4	52·2	5·4
Diethylamine	117 to 118[b]	$C_{14}H_{23}N_5O_6$	47·0	6·3	47·1	6·4
Trimethylamine	71[a]	$C_{13}H_{21}N_5O_6$	45·7	6·4	45·5	6·1
Triethylamine	91[a,b,e]	$C_{16}H_{27}N_5O_6.H_2O$	47·9	7·5	47·6	7·2
Guanidine	164 to 166[c]	$C_{11}H_{17}N_7O_6$	38·5	4·9	38·5	5·0
Glycine ethyl ester	101[a]	$C_{14}H_{21}N_5O_8$	43·8	5·4	43·4	5·4
Aniline[1]	93[a]	$C_{16}H_{19}N_5O_6$	51·0	5·0	50·9	5·0
Pyridine	85 to 86[c]	$C_{15}H_{17}N_5O_6$	49·4	4·4	49·6	4·7
2-Aminopyridine	136[a]	$C_{15}H_{18}N_6O_6$	48·0	4·8	47·6	4·8
2-Amino-4-methylpyridine	72[a]	$C_{16}H_{20}N_6O_6$	49·2	5·1	49·0	5·1
2-Amino-4,6-dimethylpyridine	127[a]	$C_{17}H_{22}N_6O_6$	50·4	5·7	50·2	5·4
2-Aminopyrimidine	118[a]	$C_{14}H_{17}N_7O_6$	44·4	4·5	44·3	4·5
2-Amino-4-methylpyrimidine	124[a]	$C_{15}H_{19}N_7O_6$	45·7	4·8	45·8	4·8
2-Aminoimidazole	163[c]	$C_{15}H_{17}N_7O_6$	42·8	4·5	42·5	4·6
2-Aminoimidazoline	184 to 186[a]	$C_{13}H_{19}N_7O_6$	42·0	5·1	42·3	5·1
Tetramethylammonium hydroxide	132[a]	$C_{14}H_{23}N_5O_6$	46·9	6·2	47·1	6·4
Hexamethonium hydroxide	114[d]	$C_{22}H_{52}N_{10}O_{12}$	49·7	7·0	50·0	6·8

[a] Recrystallised from water.
[b] Recrystallised from benzene.
[c] Recrystallised from benzene - light petroleum mixture.
[d] Recrystallised from ethanol.
[e] Decomposes when dried.

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AN IMPROVED METHOD FOR DETERMINING ^{90}Sr IN RAIN WATER

THE usual method[1] for determining ^{90}Sr of nuclear-test origin in samples of rain water involves the chemical and radiochemical purification of strontium, addition of yttrium carrier and growth of ^{90}Y over at least 16 days; yttrium is then separated, and the decay of ^{90}Y is observed.

An attempt has been made in these laboratories to separate radio-yttrium directly from the sample, thereby avoiding the storage period for the growth of ^{90}Y. It is essential for this method that the ^{90}Y is already in equilibrium with the ^{90}Sr, but the time involved in transport, particularly from the more distant sites, and in the evaporation of the samples is such that this is normally so.

An alternative approach would be to separate strontium and count the beta-activity of the source through an aluminium absorber sufficiently thick (~110 mg per sq. cm) to decrease the contribution of ^{90}Sr to negligible proportions. The growth of ^{90}Y would then be recorded, together with any ^{89}Sr present in the source. However, the absorber also decreases the count associated with the ^{90}Y, and this is undesirable for samples containing only a few micromicrocuries of ^{90}Sr; identification of ^{90}Y by decay rather than growth is therefore preferred.

The first of the two procedures described below (method A) is applicable only when the activity from short-lived rare-earth fission products is negligible. Immediately after a nuclear explosion, this condition may not be satisfied for a period whose duration will depend on a number of factors, of which the magnitude of the explosion is only one.

Method A

The sample of rain water is evaporated to small volume in the presence of nitric acid and strontium, barium, cerium, caesium and yttrium carriers. Insoluble hydroxides and carbonates are precipitated by adding solutions of sodium hydroxide and sodium carbonate, and the supernatant solution is reserved for determining ^{137}Cs. The combined precipitates are dissolved in nitric acid, and the insoluble hydroxides are precipitated by adding ammonium hydroxide. The supernatant solution is reserved for determining ^{89}Sr if required. The hydroxides are dissolved in nitric acid, and cerium is separated by precipitation as ceric iodate, this then being treated in the usual manner.[1] Yttrium hydroxide is re-precipitated and dissolved in acid, and the yttrium is then precipitated as fluoride in the presence of zirconium holdback carrier; this precipitate is dissolved, and yttrium is re-precipitated as oxalate. An yttrium oxide source is prepared, and the decay of the beta-activity is recorded both directly and also with an aluminium absorber (25 mg per sq. cm) placed between the sample and the counting-tube window. As well as ^{90}Y (half-life 64 days), the source may contain ^{91}Y and ^{147}Pm (half-lives, respectively, 58 days and 2·6 years); the last of these isotopes is a beta-emitter of relatively low energy, and ^{147}Pm activity is therefore not recorded in the measurements made with the absorber in position. Mathematical resolution of these "absorber" counts therefore gives a result for ^{90}Y, and this value is corrected for the known percentage transmission of the ^{90}Y through the aluminium absorber and also for decay from the time of the initial precipitation by ammonium hydroxide. After allowance has been made for the chemical yield of yttrium, the calculated rate of disintegration is equal to that of ^{90}Sr in the sample.

This method is particularly applicable to samples of rain water containing debris originating only from old nuclear-test explosions, when there are insignificant amounts not only of the short-lived rare-earth fission products, but also of ^{91}Y; in these circumstances, the activity recorded through the absorber is solely due to ^{90}Y. It is possible also in these circumstances to estimate the ^{147}Pm activity, use being made of the result for yttrium, together with the counts obtained directly, *i.e.*, without the absorber in position. Several samples collected in the summer and autumn of 1960 have been treated in this way.

Method B

Some modification of method A is necessary for samples that may contain significant amounts of short-lived rare-earth fission products, *e.g.*, ^{153}Sm, ^{147}Nd and ^{143}Pr (half-lives, respectively, 47 hours and 11 and 14 days). In the initial treatment, the only difference is that samarium and neodymium carriers should be added to the sample. Then, after precipitation of the yttrium and rare-earth fluorides, radiochemical and chemical purification of the yttrium is completed by ion-exchange chromatography. The yttrium and rare-earth fluorides are converted into the corresponding hydroxides, which are dissolved in dilute hydrochloric acid, and the solution is loaded on a column of the cation-exchange resin Zeo-Karb 225. Elution is carried out with 0·5 M sodium lactate at pH 2·70, individual 2-ml fractions being collected automatically. Yttrium is eluted before the rare earths and is precipitated as oxalate. An yttrium oxide source is prepared for beta-counting as described above, the decay of this source is observed, and the activity is resolved into ^{90}Y and ^{91}Y.

In the absence of short-lived fission products of samarium and neodymium, ^{147}Pm can be determined by further elution from the column, combination of the samarium, neodymium and intermediate fractions and precipitation as oxalate. After ignition of the rare earths to oxide, a source is prepared for beta-counting and the ^{147}Pm activity is recorded. Some confirmation of the identity of this radionuclide has been obtained by absorption measurements with an aluminium absorber (7 mg per sq. cm) and by the absence of significant decay over several months.

Results

Full details of the methods outlined above will be published elsewhere.[2] Table I shows examples of the results for ^{90}Sr, together with the values obtained by the usual method,[1] and four results for ^{147}Pm. These more direct approaches permit a result for ^{90}Sr to be obtained within approximately half the usual time, with no additional effort.

TABLE I

ACTIVITY FOUND IN SAMPLES OF RAIN WATER

Sample No.	Activity found in whole sample: ^{90}Sr, μμc	Activity found in whole sample: ^{147}Pm, μμc	Activity found for ^{90}Sr, by usual method, μμc
Proposed method A used—			
1	39	—	43
2	8·7	~25	10·3
3	11·5	~27	10·6
4	16·9	~41	15·8
5	15·5	—	14·9
6	23·2	—	23·5
Proposed method B used—			
7	149	—	153
8	57	~110	59

The results for ^{90}Sr in Table I form part of the information reported by the U.K. Atomic Energy Authority arising from their work on the world-wide deposition of fission products from nuclear-test explosions.[3]

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THE DETECTION OF PALLADIUM WITH *p*-NITROSODIPHENYLAMINE

DURING work on acute carbon monoxide poisoning in rats,[1] in which reduction of palladium chloride solution is used to determine carbon monoxide in blood, it was considered that *p*-nitrosodiphenylamine might be a suitable reagent for determining the excess of palladium chloride; the red complex of this reagent with palladium ions can be extracted from solutions in dilute hydrochloric acid by diethyl ether, ethyl acetate and chloroform.[2] Although the reaction was found to be too sensitive for our purpose, this Note records certain observations on the extraction of the *p*-nitrosodiphenylamine - palladium complex.

REAGENTS—

p-*Nitrosodiphenylamine reagent solution*—Dissolve 50 mg of *p*-nitrosodiphenylamine in 500 ml of 95 per cent. ethanol, and dilute to 1 litre with water.

Palladium chloride solution—Dissolve, with heating, 0·444 g of pure palladium chloride in 25 ml of 0·1 N hydrochloric acid, and dilute to 250 ml with water.

1 ml ≡ 1·06 mg of palladium.

PRELIMINARY EXPERIMENTS—

In a paper on the detection of palladium, platinum and rhodium with *p*-nitrosodiphenylamine, Ryan[2] described a procedure in which solutions containg 3 μg of palladium per ml were treated with hydrochloric acid and 1 ml of the reagent solution and, after 5 minutes, were extracted with ethyl acetate. A deep-red colour developed in the acetate layers from solutions up to 0·1 M in hydrochloric acid. Since the reaction is masked by iridium, rhodium and cobalt, an extraction procedure is of obvious value; further, it would lead to a gain in sensitivity.

In our preliminary experiments, 1-ml aliquots of the palladium solution were placed in 100-ml calibrated flasks, the requisite amount of N hydrochloric acid was added to each, and the solutions

were diluted to volume with water. Duplicate 1-ml aliquots, each containing 10·6 μg of palladium, were placed in glass-stoppered test-tubes, and 3 ml of water and then 1 ml of the reagent solution were added to each. The solutions were set aside at room temperature for 30 minutes; each solution in one series was then shaken vigorously with 5 ml of ethyl acetate and each in the other series with 15 ml of chloroform. (The difference between the volumes of the solvents used was due to the relatively low solubility of the complex in chloroform.) The results for a range of initial concentrations of acid are shown in Table I.

TABLE I

EFFECT OF ACIDITY ON EXTRACTION OF *p*-NITROSODIPHENYLAMINE - PALLADIUM COMPLEX

Each mixture tested contained 10·6 μg of palladium

Concentration of hydrochloric acid in mixture before extraction, N	Extraction with 15 ml of chloroform	Extraction with 5 ml of ethyl acetate
0·0002	Complex separates at interface	About 20 per cent. of complex extracted
0·0004	About 20 per cent. of complex extracted	About 60 per cent. of complex extracted
0·0012	About 80 per cent. of complex extracted	Complex completely extracted
0·002 to 0·016	Complex completely extracted	
0·018 to 0·1	Turbidity in aqueous layer; complex completely extractable	Turbidity in aqueous layer; complex completely extractable

DISCUSSION OF RESULTS

When the solution was initially 0·0002 N in hydrochloric acid, about 20 per cent. of the complex could be extracted with 5 ml of ethyl acetate, whereas extraction with 15 ml of chloroform resulted in separation of the complex at the interface. Ryan[2] found that ethyl acetate and chloroform gave similar results when used as extractant, but I have found that extracts of the palladium complex in ethyl acetate are much more stable than those in chloroform.

For concentrations of acid ranging from 0·018 to 0·1 N, turbid solutions resulted, although re-extraction of these solutions with ethyl acetate or chloroform gave clear red solutions. If qualitative tests for the platinum metals are to be made as described by Ryan,[2] it is therefore essential that the initial concentration of hydrochloric acid be kept within the range 0·002 to 0·01 N and not "feebly acid," as stated previously.[2] In order to prevent loss of sensitivity through use of too much solvent, the volume of ethyl acetate was kept to 5 ml; this resulted in an intense red colour when 10·6 μg of palladium were present as the *p*-nitrosodiphenylamine complex.

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PRIMARY STANDARDS IN TIN ANALYSIS

THE use of stannous solutions as primary standards in analysis is complicated by the ease with which tin is oxidised to the quadrivalent state. When tin solutions are used, they are generally prepared by dissolving either chemically pure tin metal or tin^{II} chloride dihydrate in hydrochloric acid. The resulting solutions, however, are unstable in the presence of oxygen. Moreover, dissolution of tin in hydrochloric acid is time-consuming, and most commercially available samples of tin^{II} chloride dihydrate contain appreciable amounts of tin^{IV} as impurity.[1] The need for a pure, stable and readily soluble tin^{II} compound for use as a primary standard is apparent. The work described here is a comparison of five different sources of stannous tin.

Method

Stannous and total tin were determined by Donaldson and Moser's[2] method, except that Chalmers and Thomson's[3] precision burette was used to obtain more accurate titrations. The burette was calibrated at 5-mm intervals on the micrometer head by weighing the amount of water delivered. The average volume delivered per 5 mm was 4·649 ± 0·003 ml and the standard deviation for any volume delivered was calculated to be 0·004 ml.

Blank determinations were carried out on all reagents to ensure that they contained no impurity that could affect the precision of the volumetric determination of tin.

Reagents—

Chemically pure tin—Sample containing 99·999 per cent. of tin.

Tin^{II} acetate[4]—Heat under reflux blue-black tin^{II} oxide with glacial acetic acid, filter, and concentrate the solution in a rotary evaporator. Filter, and wash the product with the minimum of water and then acetone, and purify by vacuum sublimation. The blue-black tin^{II} oxide should be prepared, under an atmosphere of oxygen-free nitrogen, by Fink and Mantell's[5] method, except that tin^{II} sulphate must be used as the starting material instead of the less satisfactory[1] tin^{II} chloride dihydrate, otherwise any chloride impurity in the final product will be sublimed along with the tin^{II} acetate. No sulphate, chloride, acetic acid, lead, copper or iron could be detected in the final sample.

Tin^{II} chloride dihydrate—Analytical-reagent grade.

Anhydrous tin^{II} chloride[6]—Heat under reflux 10 g of tin^{II} chloride dihydrate with 10 ml of acetic anhydride and 10 ml of acetic acid.

Tin^{II} sulphate—Prepared by Donaldson and Moser's[1] method.

Results

The results for six determinations of stannous and six determinations of total tin on each sample are shown in Table I. The results are quoted to 0·01 mg for statistical purposes only.

Table I

Results

Source of stannous tin	Weight of tin taken, mg	Weight of tin found, as tin^{II}, mg	Difference, mg	Weight of tin taken, mg	Weight of tin found, as total tin, mg	Difference, mg
Chemically pure tin (Sn)	99·54	99·54	0·00	149·40	149·20	−0·20
	116·92	116·95	+0·03	114·97	114·84	−0·13
	69·98	69·97	−0·01	89·60	89·50	−0·10
	108·68	108·64	−0·04	86·01	85·90	−0·11
	138·30	138·31	+0·01	54·26	54·18	−0·08
	146·72	146·68	−0·04	124·97	124·77	−0·20
Tin^{II} acetate ($Sn(CH_3COO)_2$)	118·87	118·83	−0·04	110·15	109·83	−0·32
	135·68	135·70	+0·02	70·32	70·20	−0·12
	75·20	75·17	−0·03	109·69	109·55	−0·14
	135·15	135·19	+0·04	114·19	113·95	−0·24
	143·79	143·74	−0·05	73·20	73·05	−0·15
	111·27	111·28	+0·01	82·56	82·37	−0·19
Tin^{II} chloride dihydrate ($SnCl_2.2H_2O$)	83·18	81·96	−1·22	141·60	140·75	−0·85
	86·98	85·71	−1·27	163·66	163·04	−0·62
	64·55	63·50	−1·05	66·67	66·41	−0·26
	69·18	68·03	−1·15	62·58	62·25	−0·33
	154·70	152·11	−2·59	85·69	85·21	−0·48
	62·19	61·26	−0·93	63·38	63·06	−0·32
Tin^{II} chloride ($SnCl_2$)	123·36	118·29	−5·07	153·31	150·93	−2·38
	110·30	105·57	−4·73	91·18	89·75	−1·43
	101·16	96·95	−4·21	94·89	93·29	−1·60
	109·88	105·28	−4·60	109·48	107·79	−1·69
	109·05	104·38	−4·67	110·27	108·45	−1·82
	130·08	124·80	−5·28	170·55	167·72	−2·83
Tin^{II} sulphate ($SnSO_4$)	173·15	173·91	+0·76	127·70	128·11	+0·41
	122·31	122·96	+0·65	98·64	99·02	+0·38
	117·84	118·39	+0·55	118·70	119·11	+0·41
	106·25	106·75	+0·50	123·83	124·26	+0·43
	120·29	120·83	+0·54	116·13	116·59	+0·46
	120·26	120·79	+0·53	115·72	116·16	+0·44

In each test the difference between the weight of tin found and that calculated, from the weight of tin compound taken, is given for comparison of errors. Calibrated weights were used for all weighings. The calculated observational errors[7] were found to be $\pm 0{\cdot}01$ mg for tin from weighed samples and $\pm 0{\cdot}10$ mg for tin determined volumetrically.

Discussion of results

Comparison of the differences between the weighed and calculated amounts of tin shows that only in determinations of stannous tin with chemically pure tin and with tin^{II} acetate are these differences less than the observational errors. Tin^{II} chloride and tin^{II} chloride dihydrate are obviously not suitable sources of stannous tin, whereas tin^{II} sulphate, although the most satisfactory source of tin^{II} for preparative work, is not the most satisfactory analytical standard. Determination of total tin is found to be less accurate than that of stannous tin because of the extra chemical process involved. This is shown by the determinations of total tin on chemically pure tin and on tin^{II} acetate. The presence of tin^{IV} impurity in tin^{II} chloride and tin^{II} chloride dihydrate is confirmed by the total-tin content being considerably higher than the stannous tin content; this is also responsible for the apparent improvement in results for total tin on these materials. Tin^{II} chloride and tin^{II} chloride dihydrate should not, therefore, be used as analytical standards. The slightly high results obtained with tin^{II} sulphate suggest that small amounts of oxidisable impurity are produced during its preparation. The sample used for comparison was freshly prepared, but analysis of an older sample (4 months) gave an average difference between weight taken and weight calculated of $+0{\cdot}21$ mg for stannous tin and $+0{\cdot}48$ mg for total tin. No appreciable difference was found between the results of determinations on freshly prepared and 4-month-old samples of tin^{II} acetate.

The most serious source of error in the method of analysis results from the fact that the error in standardising the ceric sulphate solution used—with resublimed arsenious oxide—is much greater than the errors involved in the use of the precision burette. A new requirement thus arises in that the primary standards used with this burette must be capable of standardisation to better than the 1 in 1000 tolerated in normal procedures. In fact, tin^{II} acetate seems to be a more satisfactory primary standard for ceric sulphate (if used as described) than is the customary arsenious oxide.

The results show that tin^{II} acetate, purified by vacuum sublimation, is a satisfactory primary standard for tin analysis. Moreover, its solubility in dilute mineral acids makes it preferable to chemically pure tin as the source of stannous tin. Because of the ease of oxidation of the tin to the quadrivalent state it is preferable to prepare the standard solutions as required, and solutions of the acetate can be obtained quickly, with complete exclusion of oxygen, and used *in situ*. Work is at present in progress on the chemistry of tin^{II} acetate and will be reported elsewhere.

One of us (W.B.S.) is grateful to the International Tin Research Council for a maintenance grant.

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THE MICRO-DETERMINATION OF SULPHUR IN ORGANIC COMPOUNDS

Various micro methods for determining sulphur in organic materials have been derived from that of Dennstedt,[1] *viz.*, the absorption on metallic silver of the oxides of sulphur formed by burning the sample in oxygen over a platinum catalyst, or from Belcher and Ingram's "empty-tube"

method.[2] In both procedures, the oxides of sulphur are absorbed on silver gauze, wire or wool, and the resulting silver sulphate is determined by gravimetric[3,4,5] volumetric[6,7,8] or colorimetric[9] methods, most of which are based on the solubility of silver sulphate in hot water.

In 1956, Ingram reviewed experience with the "empty-tube" procedure[2] and discussed its application to the determination of carbon, hydrogen and other elements. The determination of sulphur by absorption of the oxides of sulphur on silver gauze at first gave satisfactory results, but Ingram reported that low results were obtained after several combustions had been carried out, and there was evidence for reaction between the silver and the silica tubing. By enclosing the silver in a platinum cylinder to avoid contact with the silica, we have obtained satisfactory results by this method. The procedure used was similar to that described by Mitsui and Sato[10] for determining halogen by absorption on silver, and the construction of the apparatus is shown in Fig. 1. A platinum cylinder terminating in a B7 socket fits on the silica B7 cone. In use, the products of combustion, together with excess of oxygen, are drawn from the combustion furnace and, after mixing with air from the scavenging train (containing anhydrone and soda asbestos), are drawn through electrolytic silver contained in the platinum cylinder. The increase in weight of the cylinder and its contents is ascribed to the formation of silver sulphate, and the sulphur content of the original substance can thus be calculated.

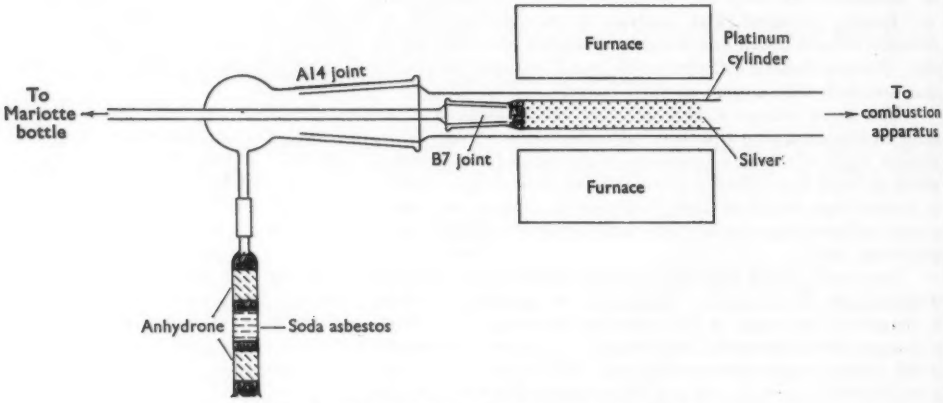

Fig. 1. Apparatus used for determining sulphur

Combustion was at first carried out in a tube containing platinum catalyst. The results were satisfactory, and the use of the "empty-tube" method was therefore examined in an attempt to decrease the time necessary for combustion. With the silver filling at temperatures between 650° and 700° C, as recommended by Ingram, results were low by 0·5 to 1·0 per cent., *e.g.*, for sulphonal (theoretical content of sulphur 28·10 per cent.) the results were—

Weight of sample, mg	5·363	4·856	3·209	3·846	4·483	4·317
Sulphur content found, % ..	27·80	28·15	27·75	27·05	27·40	27·45

Further, after several determinations, a yellow stain was observed on the silica tubing. When the temperature was maintained between 500° and 550° C, results were satisfactory. Recently, we have used electrolytic silver, as recommended by Mitsui and Sato,[10] and have obtained equally good results (see Table I).

Method

Apparatus—

A combustion tube of the type recommended[2] for determining carbon and hydrogen by the rapid-combustion procedure was used in our experiments. A small furnace was used to heat the silver contained in the platinum cylinder. The latter was seamless and had an over-all length of 10 cm (inclusive of the B7 socket) and an internal diameter of 7 mm; the wall thickness was 0·01 inch and the empty cylinder weighed 12 g.

TABLE I

SULPHUR CONTENTS FOUND WITH USE OF ELECTROLYTIC SILVER AT 500° C

Compound	Weight of sample, mg	Sulphur content found, %	Calculated sulphur content, %
Sulphonal	4·609	27·95	28·10
	4·150	28·15	
	4·532	28·05	
	3·816	28·00	
	4·997	28·05	
	3·582	28·10	
	3·342	28·05	
	4·433	28·00	
	4·584	28·30	
	4·730	28·05	
Thiourea	3·686	41·95	42·10
Phenylthiourea	4·134	21·10	21·05
$\beta\beta'$-bis(Carboxymethylmercapto)diethyl ether	4·250	25·10	25·20
Phenylsulphone acetic acid	4·259	16·05	16·00

PROCEDURE—

Boil the platinum cylinder in 50 per cent. nitric acid, wash with distilled water, and ignite at 1000° C for 30 minutes. Place a small wad of silver wool in the cylinder, and tamp it firmly down with two pieces of glass rod so that the wool is located at the neck of the joint. Prepare some electrolytic silver wool,[10] pour it, together with the electrolyte solution, on to the silver wool pad, and apply gentle suction to consolidate the metal. Add sufficient silver (about 3 g) to fill the cylinder from the joint to within 2 cm of the other end. Wash out the electrolyte with distilled water, and remove the latter by washing with ethanol and ether. Dry the tube and its contents in an oven at 110° C, and, finally, heat at 500° C for 30 minutes.

Pass oxygen into the combustion tube at 25 to 30 ml per minute; no purification of the oxygen is necessary. Adjust the suction at the exit of the combustion tube (by lowering the arm of the Mariotte bottle) to give a flow of 50 ml per minute. Remove the A14 joint, attach the platinum cylinder, and replace the joint. After 5 minutes, "burn out" the exposed part of the combustion tube, and, after a further 5 minutes, turn off the suction. Remove the platinum cylinder, lay it across a large brass block, and place both beside a microbalance for 10 minutes. Weigh 3 to 8 mg of sample in a micro platinum boat. Allow the cylinder to rest on the balance stirrups for 10 minutes, and then weigh it to the nearest microgram. Re-connect the cylinder to the combustion apparatus, and introduce the sample in the usual manner. Turn on the suction, and allow 5 minutes for the cylinder and its contents to attain 500° C. Burn the sample as for the determination of carbon and hydrogen, taking 4 to 6 minutes for the combustion, and sweep out for 9 to 11 minutes. Remove the cylinder, cool, and weigh it as before. Calculate the sulphur content of the sample from the equation—

$$\text{Sulphur content, \%} = \frac{\text{Increase in weight of cylinder and filling, mg} \times 0{\cdot}334 \times 100}{\text{Weight of sample, mg}}$$

This work was carried out in the branch of the Superintendent of Analytical Chemistry, Chemistry Division. I thank Mr. P. N. S. Ibbetson for assistance in the practical work.

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U.K. ATOMIC ENERGY AUTHORITY
ATOMIC WEAPONS RESEARCH ESTABLISHMENT
ALDERMASTON, BERKS.

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Book Reviews

ORGANO-METALLIC COMPOUNDS. By G. E. COATES, M.A., D.Sc., F.R.I.C. Second Edition. Pp. xiv + 366. London: Methuen & Co. Ltd.; New York: John Wiley & Sons Inc. 1960. Price 45s.

In order to include many of the important developments in the field of organo-metallic compounds, Professor Coates has found it necessary to revise and extend his well known monograph. Despite the addition of much new material on compounds of the alkali metals, boron, tin and the transition elements and greatly extended discussions of the many interesting valency problems raised by, *e.g.*, sandwich compounds and pi-complexes of various kinds, the author has succeeded in keeping the whole subject in perspective and in writing an authoritative and thoroughly readable book.

Organo-metallic compounds, apart from Grignard reagents, certain boro- and alumino-hydrides and bulky precipitating ions, such as $[B(C_6H_5)_4]^-$ or $[As(C_6H_5)_4]^+$, have not been greatly exploited in analysis, and this aspect is not dealt with in this book. The statement (p. 163) that thallium is unique in forming a cyclopentadienyl complex, C_5H_5Tl, when the components are shaken in *aqueous* solution is certainly correct. The statement that the product is moderately soluble in most polar solvents immediately suggests a remarkable solvent-extraction technique which would be absolutely specific for this element! Unfortunately, experiment does not confirm the alleged solubility.

H. IRVING

PROCEEDINGS OF THE SYMPOSIUM ON THE CHEMISTRY OF CO-ORDINATION COMPOUNDS, AGRA, INDIA, FEBRUARY 7TH & 8TH, 1959. Part 1: pp. vi + 148; Part 2: pp. iv + 203; Part 3: pp. vi + 302 + vi. Allahabad, India: National Academy of Sciences. 1960. Price (Part 1) Rs. 15.00; (Part 2) Rs. 25.00; (Part 3) Rs. 35.00; (all three Parts) Rs. 75.00.

These three volumes report the proceedings at a symposium held at the University of Agra on February 7th and 8th, 1959. Part 1 begins with an interesting presidential address by Professor Ray and continues with an important and fully documented review of Indian contributions to the study of co-ordination chemistry (64 pages) and 8 papers of a miscellaneous character. Part 2 contains 28 papers dealing with valency problems, stereochemistry and structure and 6 papers dealing with techniques and methods of investigation. Part 3 contains 32 papers on reactions, stability and thermodynamic considerations, 2 on the stabilisation of valency states, 13 dealing with analytical applications and 3 unclassified.

It must be admitted that the quality and style of the papers vary enormously from sophisticated treatments of band spectra and thermodynamics to the frankly pedestrian. Some of the contributions appear to be mere abstracts of potential papers, others are given at length and are well illustrated and documented; this is especially true of some of the review articles. It would appear that the organisers did not stipulate that the contributions should be original, for quite a few (more especially from European contributors) had first seen the light of day at previous conferences.

Readers of *The Analyst* will naturally be most interested in the 87 pages devoted to analytical applications of co-ordination compounds. Several papers dealt with the extensive original researches carried out (especially in India) on the analytical potentialities of various organic reagents, notably oximes, hydroxamic acids, polyphenols and derivatives of thiourea and dithio-oxamide. Others dealt with the gravimetric determination of copper, the analytical chemistry of thorium, the determination of free acid in the presence of hydrolysable cations, metallochromic indicators and paper chromatography. Nothing very novel or useful could be gleaned from this miscellany. Certainly, much of the data on the behaviour of new organic reagents for metals could form the basis for further work; but it is only right to comment that no attempts are made to assess whether or not the new reagents show any advantages over those already in use, for problems of interferences and practical details do not come in for much attention.

The editors of so large a symposium must have had an exceptionally difficult task in collating manuscripts from authors of so many different nationalities and interests and spread so widely over the earth's surface. Despite the very large number of errors, many in printing, but some due clearly to errors in the original manuscripts, the Convenor and the printers have done a very good job, and these three volumes testify to the extent and quality of the research work carried out in India and to the lively interest taken there in the still rapidly expanding field of co-ordination chemistry.

H. IRVING

TOXICOLOGY AND BIOCHEMISTRY OF AROMATIC HYDROCARBONS. By HORACE W. GERARDE, M.D., Ph.D. Pp. xiv + 329. Amsterdam, London, New York and Princeton: Elsevier Publishing Company; London: D. Van Nostrand Co. Ltd. 1960. Price 30s.

This book is the third in a series of Elsevier monographs on toxic agents. It is packed full of interesting bits of information about aromatic hydrocarbons, such as economic importance, sources, uses in industry, analytical procedures, physical properties, etc. These are, however, merely the fringes of the main body of the book, which deals with the toxicology and biochemistry of the aromatic hydrocarbons. The book is divided into two parts, the first part dealing generally with the subject and covering about a third of the text, and the second part dealing in more detail with individual aromatic hydrocarbons of industrial importance, beginning with benzene and passing through mono-, di- and polyalkylbenzenes to di-, tri- and polycyclic hydrocarbons. There are useful tables listing carcinogenic and non-carcinogenic hydrocarbons in the chapter on polycyclic hydrocarbons.

"The purpose of the book is to assemble in one volume information primarily of interest to the physician, industrial hygienist and toxicologist," and therefore data of direct interest to the analyst, *i.e.*, analytical procedures, are compressed into about ten pages, although the last chapter, on the composition of solvents, fuels and lubricants, must also be of value to analysts. In the section on analytical procedures, emphasis is placed on physical methods, such as the combustible-gas indicator, refractive index, ultra-violet and infra-red absorption and polarography. Chemical methods are referred to in about two pages. It should be pointed out that the analytical procedures are those that can be applied to air (air pollution), blood, urine and tissues, since the book deals with the biological aspects of aromatic hydrocarbons.

This small-sized book is nicely produced in semi-stiff paper covers, and the figures and formulae are easy to understand. The 59 tables are mines of information, and the book contains an interesting appendix consisting of a glossary of the terms used in the petroleum industry. For the analyst interested in toxicology, this book can be recommended because it contains so much useful information in a relatively small space.

R. T. WILLIAMS

ADVANCES IN POLAROGRAPHY: PROCEEDINGS OF THE SECOND INTERNATIONAL CONGRESS HELD AT CAMBRIDGE, 1959. Edited by IAN S. LONGMUIR. Volume 1. Pp. xvi + 1–407. Volume 2. Pp. x + 408–803. Volume 3. Pp. vii + 804–1204. Oxford, London, New York and Paris: Pergamon Press (Symposium Publications Division). 1960. Price (3 volumes) £15.

The 103 full-length papers included in these three volumes were contributed by polarographers from 19 countries to the Second International Congress on Polarography, held at Cambridge in August, 1959. The papers range from authoritative reviews of published work to interim accounts of original work still in progress and cover all aspects of polarography, including training of students, fundamental theory, instrumentation and analytical applications in fields ranging from metallurgy to medicine. With the exception of ten in German and eight in French, all the papers are printed in English. The formal discussion after each paper has been included.

The Cambridge Congress met in rather different circumstances from the First International Congress, held at Prague in February, 1951. Although papers from all over the world were submitted to and published in the Proceedings of the Prague Congress, only polarographers from Eastern Europe were able to attend and participate in the Congress meetings. At Cambridge, distinguished polarographers from East and West were able to meet and freely discuss their results. The unfortunate absence through ill-health of Professor J. Heyrovský, the founder of polarography, provided the only major disappointment; his inaugural address on oscillographic polarography was read by his son.

A comparison of these three volumes with those issued after the Prague Congress reveals an interesting picture of the development of polarography during the intervening decade. Progress on the inorganic side has been dominated by the development of new analytical applications in the field of atomic energy; this has encouraged spectacular improvements in instrumentation and has led to better methods for removing interfering substances. On the organic side, there has been a greater interest in reduction mechanisms and a wider use of non-aqueous solvents. Papers in these volumes, largely by British authors, describe new applications of the cathode-ray polarograph and discuss the scope and limitations of square-wave and pulse polarography. A stimulating

article on alternating-current polarography by Barker, the inventor of the square-wave and pulse polarographs, considers the analytical possibilities of radio-frequency polarography. Czech and Russian authors have contributed papers on oscillographic polarography.

The articles on organic and biological applications are of varied interest. Given and Peover's critical account of the polarography of organic substances in organic solvents and of the application of the method to the study of coal deserves special notice. Other papers deal with the determination of oxygen in respiring biological fluids, the analysis of blasting explosives, the influence of substituents on the reduction of aliphatic and heterocyclic compounds, the direct determination of 2-ethyl-4-thiocarbamoylpyridine in biological fluids and the morphology of the Brdička catalytic wave.

There can be few analysts who will not find something of interest in these volumes; they can be recommended without reservation as substantial supplements to the standard monographs on polarography. The volumes are well produced, but, at their price, there will unfortunately be few analysts able to afford personal copies. J. E. PAGE

X-RAY ABSORPTION AND EMISSION IN ANALYTICAL CHEMISTRY: SPECTROCHEMICAL ANALYSIS WITH X-RAYS. By H. A. LIEBHAFSKY, H. G. PFEIFFER, E. H. WINSLOW and P. D. ZEMANY. Pp. x + 357. New York and London: John Wiley & Sons Inc. 1960. Price $13.50; 108s.

X-Ray spectrochemical analysis has had an interesting, though somewhat chequered, history. Almost from the moment of discovery of X-rays, and certainly after the electromagnetic character of the radiation was confirmed, X-ray spectral lines could have been used for analytical purposes. Moseley's classical measurements of X-ray wavelengths between 1913 and 1915 pointed the way to element identification and determination. But such pointers do not appear to have been followed up at all systematically until about 1930, when Laby and his collaborators in Australia made an extended study of the practical possibilities. Even then the engineering difficulties involved, because it was still necessary to excite the X-ray spectra by electrons inside continuously evacuated demountable generators, appear to have discouraged others from an immediate exploitation of Laby's work. It was not until after the end of the second World War that it was realised that advances in instrumentation had occurred, which provided the basis for wide application. Geiger counters and other types of detector devices had become available and permitted measurement to be made of the relatively weak X-ray spectra emitted by a specimen struck by a primary X-ray beam outside the actual generator.

This historical background is made apparent in the text of the book. The authors are immensely optimistic about future prospects. Indeed, they venture to declare that "all spectroscopists should become familiar with the X-ray methods described" and that "X-ray emission spectrography will gain so rapidly upon the optical that the variety of analyses being done by each method will have become comparable before 1970" (pages 237–238). There is no denying that the authors are most able advocates of this thesis. The reader is left in little doubt that in certain fields, particularly when an analytical method that will give automatic control of a manufacturing process is desirable, the X-ray spectrochemical technique has a high chance of filling the gap.

The book is deliberately written for the analytical chemist. In the early part, basic information about the physics of X-ray emission and the nature of X-ray spectra is clearly presented, in order that the reader previously unacquainted with the subject may understand the principles involved. The various forms of detector and counter are described and compared, and guidance is given to selection for different applications.

There are two chapters on X-ray absorptiometry, with polychromatic and monochromatic beams, respectively, and another chapter discusses film-thickness determination by X-ray emission methods. So far as the analyst is concerned, however, perhaps the most important are the later chapters, from the seventh onwards, which are devoted to X-ray emission spectrography. These form the backbone of the book. The exposition is clear and direct. The many problems involved in developing satisfactory quantitative methods are frankly discussed, and the accounts of practical applications are particularly convincing because for illustrations the authors are able to draw freely upon their own wide experience. Representative X-ray spectrographs are described, covering a range from relatively simple laboratory apparatus to fully automatic multi-channel equipment for analytical control. Chapter 10 contains an excellent appraisement of the reliability

of the X-ray method. The careful analysis of errors in this chapter is worthy of study by many others besides those engaged in the X-ray spectrochemical field.

Only a very brief description of the X-ray emission microprobe development is given. In view of the growing importance of this technique, the reviewer would have preferred a fuller account, in place of the special fringe topics (including absorption and emission of gamma rays, X-ray microscopy and histochemical analysis) that form the subject matter of the last chapter.

There are several tables in appendixes. These include spectral wavelength data, absorption edges, mass-absorption coefficients and so on. The book is excellently printed and bound. It can be thoroughly recommended, not only to analytical chemists, but also to other chemists who wish to become more informed on a subject of growing practical importance.

H. P. Rooksby

Toxicology: Mechanisms and Analytical Methods. Edited by C. P. Stewart and A. Stolman. Volume I. Pp. xviii + 774. New York and London: Academic Press Inc. 1960. Price $22.00; 157s. 6d.

Until recent years there was a dearth of books about methods of analysis as applied to toxicology. Several comprehensive volumes on the subject have recently been published in this country and in the U.S.A. This book is edited by a combination from both countries—C. P. Stewart is at the Department of Clinical Chemistry, Edinburgh, and A. Stolman is at the Toxicological Service Division of the Connecticut Department of Health. The editors have also chosen an impressive list of contributors from both sides of the Atlantic.

The first part of the book deals with the absorption, distribution and excretion of poisons and their metabolites, and the second part is concerned with general techniques for the identification, separation and determination of these substances in toxicological material. From the analyst's point of view, the second part is of more interest, the first portion being useful as a source of reference. The analytical portion opens with a chapter by Dr. Curry, of Harrogate Forensic Laboratory, outlining the method advocated for a systematic search for an unknown poison in viscera. This is very comprehensive and is probably the best technique as yet published for such an investigation.

This is followed by chapters on the isolation and separation of poisons in biological material by all the known chemical methods, including distillation, micro-diffusion, chromatography (with its various techniques), ion-exchange resin procedures, paper ionophoresis and counter-current distribution. The various spectrographic and spectroscopic applications to the subject are described, as well as X-ray diffraction analysis. In addition, there is a chapter on optical-crystallographic methods involving use of a polarising microscope. This method of identification of minute quantities of material has been developed to a great extent in the last five years by the author of this chapter, and many of the constants are given in the tables to assist the reader in his task.

It will be seen that the volume is most comprehensive in approach. It has also been prepared and is presented with great care as to accuracy in detail, in a manner easy to follow. Many of the methods are outside the scope of all but specialised laboratories, but the majority of techniques described are generally applicable. It does emphasise, however, that in all branches of applied analysis, the specialisation in up-to-date techniques, including advanced instrumentation, is essential if the subject is to be treated with the exactitude and speed now demanded.

R. F. Milton

Publications Received

The Radiochemistry of Antimony. By William J. Maeck. Pp. vi + 54. Washington, D.C.: National Academy of Sciences—National Research Council. 1961. Price 50 cents. *Nuclear Science Series: NAS—NS-3033.*

The Radiochemistry of Titanium. By Chong Kuk Kim. Pp. vi + 23. Washington, D.C.: National Academy of Sciences—National Research Council. 1961. Price 50 cents. *Nuclear Science Series: NAS—NS-3034.*

THE RADIOCHEMISTRY OF CESIUM. By H. L. FINSTON and M. T. KINSLEY. Pp. vi + 68. Washington, D.C.: National Academy of Sciences—National Research Council. 1961. Price 75 cents.
Nuclear Science Series: NAS—NS-3035.

INVESTIGATION OF RATES AND MECHANISMS OF REACTIONS. Part I. Edited by S. L. FRIESS, E. S. LEWIS and A. WEISSBERGER. Second Edition. Pp. xii + 702 + Index. New York and London: Interscience Publishers Inc. 1961. Price $23.50.

THE EXTRA PHARMACOPOEIA (MARTINDALE): SUPPLEMENT 1961. Published by direction of the Council of The Pharmaceutical Society of Great Britain. Pp. xii + 315. London: The Pharmaceutical Press. 1961. Price 32s. 6d.

HANDBOOK OF ORGANOMETALLIC COMPOUNDS. By HERBERT C. KAUFMAN. Pp. iv + 1546. Princeton, N.J., New York, Toronto and London: D. Van Nostrand Company Inc. 1961. Price 169s.

PRINCIPLES AND APPLICATIONS OF PAPER ELECTROPHORESIS. By CH. WUNDERLY. Pp. xii + 253. Amsterdam, London, New York and Princeton: Elsevier Publishing Company; London: D. Van Nostrand Company Ltd. 1961. Price 26s.

AN INTRODUCTION TO THE ANALYSIS OF SPIN-SPIN SPLITTING IN HIGH-RESOLUTION NUCLEAR MAGNETIC RESONANCE SPECTRA. By JOHN D. ROBERTS. Pp. xii + 116. New York: W. A. Benjamin Inc. 1961. Price $4.95.

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Monographs for Teachers No. 5.

GAS CHROMATOGRAPHY ABSTRACTS 1960. Edited by C. E. H. KNAPMAN; Assistant Editor C. G. SCOTT. Pp. xii + 200. Sponsored by The Gas Chromatography Discussion Group (Associated with the Hydrocarbon Research Group of the Institute of Petroleum). London: Butterworths Publications Ltd. 1961. Price 42s.

TITRIMETRIC ANALYSIS: AN INTRODUCTORY COURSE. By NORMAN B. B. JOHNSTONE, B.Sc., F.R.I.C., and THOMAS C. DOWNIE, B.Sc., Ph.D., F.R.I.C. Pp. 186. London: University of London Press Ltd. 1961. Price 13s. 6d.

THE RADIOCHEMISTRY OF GOLD. By J. F. EMERY and G. W. LEDDICOTTE. Pp. vi + 34. Washington, D.C.: National Academy of Sciences—National Research Council. 1961. Price 50 cents.
Nuclear Science Series: NAS—NS-3036.

LIQUID-LIQUID EXTRACTION WITH HIGH-MOLECULAR-WEIGHT AMINES. By FLETCHER L. MOORE. Pp. vi + 83. Washington, D.C.: National Academy of Sciences—National Research Council. 1960. Price $1.00.
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PROGRESS IN ORGANIC CHEMISTRY. Volume 5. Edited by J. W. COOK, D.Sc., F.R.S., and W. CARRUTHERS, Ph.D. Pp. viii + 172. London: Butterworths Publications Ltd. 1961. Price 50s.

RADIOCHEMISTRY. Scientific Translation Editor Dr. A. G. MADDOCK, Sc.D., Ph.D. Vol. I. No. 1. Pp. ii + 183. Oxford, London, New York and Paris: Pergamon Press. 1960. Annual Subscription rate £15 ($40). Single Issues £8 ($24).

Errata

AUGUST, 1961, ISSUE, Note by F. J. Griffin and C. B. Casson, p. 544, Table I, 4th column, 5th line. *For* "0·125" *read* "0·0125".

IBID., p. 544, Table I, 4th column, 6th line. *For* "0·466" *read* "0·0466".

IBID., p. 544, last paragraph, 3rd line. *For* "0·341" *read* "0·0341".